Alberta Health

ALBERTA CYANOBACTERIA BEACH MONITORING

2010–2013

September

2014

Alberta Government

For more information contact:
Health Protection Branch
Alberta Health
P.O. Box 1360, Station Main
Edmonton, Alberta, T5J 1S6
Telephone: 1-780-427-1470

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EXECUTIVE SUMMARY

Harmful blue-green algae (toxic cyanobacteria) blooms in surface water are prevalent in Alberta. The presence of blue-green algae in recreational water causes unpleasant aesthetics. Exposure to some toxin-producing blue-green algae may pose potential health risks to public. There have been increased public awareness and health concerns as a result of increased research over the past 20 years, recent monitoring efforts, as well as the general public becoming educated on the matter.

In 2010 and 2011, Alberta Health Services initiated a cyanobacteria monitoring program for shallow water adjacent to beaches and issued public health advisories based on visual inspection. The findings revealed that microcystins (MCYSTs), one group of toxins produced by cyanobacteria, dominate in Alberta's lakes and reservoirs. In order to inform the residents to safely use public beaches and implement better public health management, Alberta Health and Alberta Health Services along with other governmental departments and public health laboratories conducted the program of Alberta Cyanobacteria Beach Monitoring for Public Health in 2012 and 2013.

The objectives of this program are to :

1. establish and maintain an integrated, participatory process for responding to and managing public health issues relating to harmful blue-green algae blooms,
2. establish communication strategies across government and for the public,
3. provide scientific evidence to support development of public health advisories,
4. characterize cyanobacteria and microcystin toxin in recreational water adjacent to beaches and in fish in terms of levels, and spatial and time distribution,
5. build the capacity of Alberta public health laboratory network for monitoring cyanobacteria, and
6. validate laboratory methods for supporting implementation of the Guidelines for Canadian Recreational Water Quality (Cyanobacteria and their Toxins) (GCRWQ).

The findings are that :

1. collaborative and effective communication processes were established among relevant governmental departments and AHS for efficient public health management,
2. either the density of total cyanobacterial cells or MCYST levels exceeded the GCRWQ values for the lakes under advisories,
3. MCYST-producing cyanobacteria species were dominant in most lakes,
4. cyanobacterial blooms peaked in late August and September in most lakes,

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5. most GCRWQ -exceeding cyanobacteria blooms occurred in the northern, central and Edmonton zones,
6. MCYST levels exceeded the GCRWQ values in some lakes, but were not consistently associated with the elevated cell density in most cases,
7. MCYSTs were not detected in fish muscle samples,
8. MCYST synthase gene E determined by qPCR method was a good predictor for cyanobacterial blooms in some lakes, and
9. five laboratory methods for detecting cyanobacterial cell density and measuring MCYST levels are valid and acceptable in terms of QA/QC standards, reproducibility, reliability, sensitivity and specificity.

In conclusion, the findings indicate that :

1. a visual inspection method for cyanobacterial blooms is an effective practice in terms of timely communicating with public,
2. cell counting is a useful method for determining extent and types of species of harmful blue-green algae blooms to support issuing public health advisories,
3. the screening methods (PPI and ELISA) and confirmatory method (LC-MS/MS) add value to assess the effectiveness of risk management practices after issuing public health advisories, and
4. collaborative and effective communication approaches among stakeholders are good practice for risk management.

The recommendations are to

1. continue routine monitoring for cyanobacteria, including confirmatory testing,
2. continue the shared approach for sample collection with Alberta Health Services, Alberta Centre for Toxicology, Alberta Environment and Sustainable Resource and Development, and Alberta Tourism, Parks and Recreation,
3. select priority lakes for systematic weekly monitoring where possible (May – Oct),
4. monitor new lakes with cyanobacterial (blue-green algae) blooms, and
5. continue science-based monitoring program to improve advisory practice and communicate specific risks to an interested and informed public.

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ACKNOWLEDGEMENTS

Working Group

Environmental Health, Alberta Health Services

Health Protection, Alberta Health

Office of the Chief Medical Officer of Health, Alberta Health

Alberta Centre for Toxicology

Biological Sciences, University of Alberta

Laboratory Medicine and Pathology, University of Alberta

Water Policy Branch, Alberta Environment and Sustainable Resource and Development

Fish and Wildlife Policy, Alberta Environment and Sustainable Resource and Development

Science Advisory Committee

Dr. Ron Zurawell	Environment and Sustainable Resource and Development
Dr. Rolf Vinebrooke	University of Alberta
Dr. Steve Hrudey	University of Alberta (Professor Emeritus)
Dr. Stephan Gabos	University of Alberta
Dr. David Kinniburgh	Alberta Centre for Toxicology
Dr. Xiaoli Pang	University of Alberta

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ABBREVIATION

ACFT	Alberta Centre for Toxicology
ACMPPH	Alberta Cyanobacteria Monitoring Program for Public Health
ADDA	a unique ß-amino acid
AESRD	Alberta Environment and Sustainable Resource and Development
AHS	Alberta Health Services
ATPR	Alberta Tourism, Parks and Recreation
CAD	collision-assisted dissociation
Ct	cycle threshold
CV	coefficient of variation
DSOP	Departmental Standard Operating Procedures
ELISA	enzyme-linked immunosorbent assay
FNR	false negative rate
FPR	false positive rate
GCRWQ	Guidelines for Canadian Recreational Water Quality
LC-MS/MS	liquid chromatography linked tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MC-LF	microcystin-LF
MC-LR	microcystin-LR
MC-LW	microcystin-LW
MC-RR	microcystin-RR
MC-YR	microcystin-YR
***mcy*E**	microcystin synthase gene E
MCYST	microcystin
∑MCYST	total microcystin
ME	matrix effect
MRM	multiple reaction monitoring
NPV	negative predictive value
OCMOH	Office of the Chief Medical Officer of Health
PPI	protein phosphatase inhibition assay
PPV	positive predictive value
PSA	primary secondary amine
QA/QC	quality assurance and quality control
QC	quality control
qPCR	quantitative polymerase chain reaction
RRT	relative retention time
RT	retention time
SAC	Science Advisory Committee

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Overview

Harmful blue-green algae (toxic cyanobacteria) blooms in surface water are prevalent in Alberta. The presence of blue-green algae in recreational water causes unpleasant aesthetics. Exposure to some toxin-producing blue-green algae may pose potential health risks to public. There have been increased public awareness and health concerns as a result of increased research over the past 20 years, recent monitoring efforts, as well as the general public becoming educated on the matter.

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Part I

Validation of Laboratory Methods

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One of the objectives in Alberta Cyanobacteria Beach Monitoring Program is to validate currently used laboratory methods for determining cyanobacteria and MCYSTs in beach water and fish. In 2011-2013, three MCYST testing methods were conducted for beach water samples. In 2012 and 2013, cell counting and qPCR were added into the program.

The objectives of the Part I project are to:

1. validate five laboratory methods for determining cyanobacteria density and MCYST levels in beach waters,
2. develop and validate LC-MS/MS method to detect MCYSTs in fish muscles, and
3. validate newly developed qPCR method for establishing a good indicator.

The validation of laboratory methods focused on public health risk management by using the Guidelines for Canadian Recreation Water Quality (Cyanobacteria and their Toxins) (QCRWQ):

1. 100,000 cells/mL for recreational water users, and
2. 20 μg MCYST/L for children under four years old.

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1. Methods and Materials

1.1 Beach Water Samples

1.1.1 Sampling Locations

Water samples were collected from Alberta recreational beaches in 2010, 2011, 2012 and 2013. The numbers of lakes were 39 in 2010, 33 in 2011, 47 in 2012 and 49 in 2013. The list of lakes is showed in Appendix A.

1.1.2 Field Collection

Water samples were collected by AHS public health officers, trained students and consultants according to the AHS Department Standard Operating Procedure and the Recreational Beach Water Monitoring Handbook. Most sampling activities were conducted between June and September each year.

General beach conditions were recorded, including air temperature, wind direction, rainfall and sky conditions (sunny, cloud and rain). Ten water samples were collected along the length of a beach in a manner similar to that shown in Figure 1. The depth of water for obtaining a sample was 1 meter.

Figure 1 Sampling Spots in a Beach

A 20-cm rigid plastic tube ('wine thief') fitted with a one-way valve was used for collecting a column of water. Water was added to a 'composite' bucket between each point by depressing a valve on the bottom end of the tube within the bucket. Once ten samples were collected, the 10-point composite sample was mixed in the pail and poured into 3 sample bottles. The bottle was immediately wrapped in foil or put in the cooler to prevent photolysis of cyanobacteria and microcystin. Samples were refrigerated and then frozen prior to shipping.

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Subsamples were shipped by Purolator Courier on dry ice (Praxair) in styrofoam shipping coolers from Edmonton to the Alberta Centre for Toxicology (ACFT) on a weekly or biweekly basis for analysis.

Field procedures are showed in Figure 2.

1.1.3 Sample Information

Information on sample size and laboratory analysis methods for water samples is summarized in Table 1.

Table 1 Summary of Sample Size

Year	Cell Count	*mcy*E by qPCR	PPI	ELISA	LC-MS/MS	Total
2010	-	-	544	-	-	544
2011	-	-	479	98	103	479
2012	136	678	758	277	752	762
2013	494	599	608	261	315	612
Total	**630**	**1277**	**2389**	**636**	**1170**	**2397**

1.1.4 Visual Inspection Method

Water color was determined visually as green and blue-green. Prior to wading into the water, photographs of the swimming area were taken to capture the color and clarity of the water to compare with the water analysis results. The visual inspection was to determine whether

1. the bottom of the lake was clearly visible at approximately 30 cm depth along the shore line,
2. note any distinct green or blue-green discolouration of the water,
3. note the transparency,
4. note if cyanobacteria can be seen as green or blue-green streaks on the surface, or as accumulations in bays and along shorelines, and
5. note the extent areal coverage of the green or blue-green surface scums.

The examples of green and green-blue colors of water are illustrated in Figure 3 (Ron W. Zurawell, AESRD, 2013).

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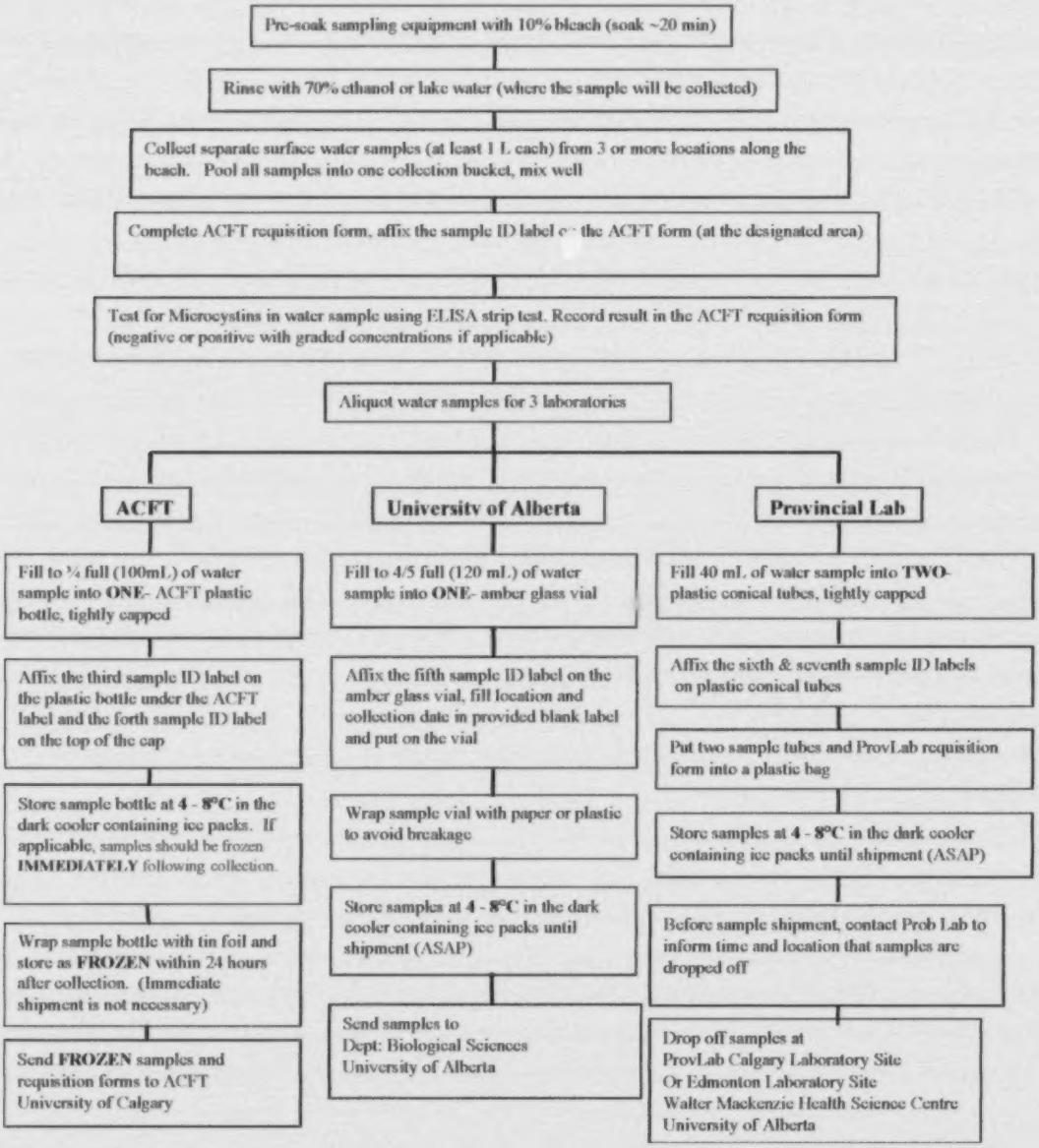
Pre-soak sampling equipment with 10% bleach (soak ~20 min)
Rinse with 70% ethanol or lake water (where the sample will be collected)
Collect separate surface water samples (at least 1 L each) from 3 or more locations along the beach. Pool all samples into one collection bucket, mix well
Complete ACFT requisition form, affix the sample ID label on the ACFT form (at the designated area)
Test for Microcystins in water sample using ELISA strip test. Record result in the ACFT requisition form (negative or positive with graded concentrations if applicable)
Aliquot water samples for 3 laboratories
ACFT
Fill to ¼ full (100mL) of water sample into ONE- ACFT plastic bottle, tightly capped
Affix the third sample ID label on the plastic bottle under the ACFT label and the forth sample ID label on the top of the cap
Store sample bottle at 4 - 8°C in the dark cooler containing ice packs. If applicable, samples should be frozen IMMEDIATELY following collection.
Wrap sample bottle with tin foil and store as FROZEN within 24 hours after collection. (Immediate shipment is not necessary)
Send FROZEN samples and requisition forms to ACFT University of Calgary
University of Alberta
Fill to 4/5 full (120 mL) of water sample into ONE- amber glass vial
Affix the fifth sample ID label on the amber glass vial, fill location and collection date in provided blank label and put on the vial
Wrap sample vial with paper or plastic to avoid breakage
Store samples at 4 - 8°C in the dark cooler containing ice packs until shipment (ASAP)
Send samples to Dept: Biological Sciences University of Alberta
Provincial Lab
Fill 40 mL of water sample into TWO- plastic conical tubes, tightly capped
Affix the sixth & seventh sample ID labels on plastic conical tubes
Put two sample tubes and ProvLab requisition form into a plastic bag
Store samples at 4 - 8°C in the dark cooler containing ice packs until shipment (ASAP)
Before sample shipment, contact Prob Lab to inform time and location that samples are dropped off
Drop off samples at ProvLab Calgary Laboratory Site Or Edmonton Laboratory Site Walter Mackenzie Health Science Centre University of Alberta

Figure 2 Field Collection Procedures

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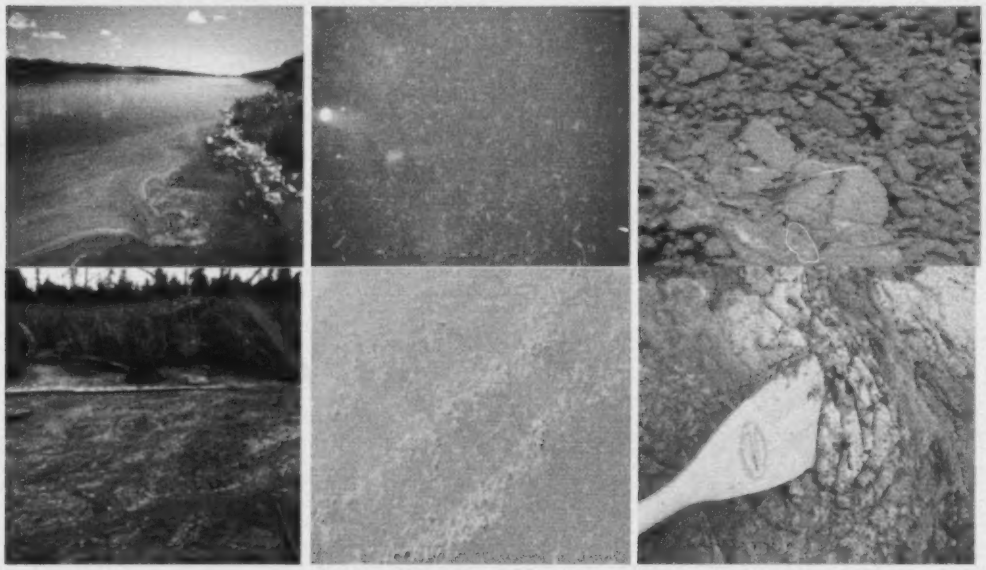

Figure 3 Blue-green Colors in Some Lakes, Alberta (photos courtesy of R. Zurawell)

1.1.5 Laboratory Methods

Three different assessments were included in the monitoring program:

1. quantifying total cyanobacteria density
    - cell counting,
2. quantifying MCYST levels
    - protein phosphatase inhibition assay (PPI)
    - enzyme-linked immunosorbent assay (ELISA)
    - liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

    and
3. quantifying MCYST synthase gene E (*mcy*E)
    - quantitative Polymerase Chain Reaction (qPCR)

1.1.5.1 Cell Counting

The cell accounting was conducted at the Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

The presence of cyanobacteria was directly examined and quantified by a trained phycologist using visible light microscopy. The method used to quantify

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preserved (Lugol's solution) phytoplankton samples was developed by the Utermöhl (Utermohl 1958) as modified by Nauwerck (Nauwerck 1963). This method involved settling a known volume of preserved sample into a counting chamber. The volume of subsample settled dictated the density of phytoplankton. After settling, a smaller volume of sample was resettled if the density of plankton was too great. This increased the accuracy in detecting those rare species, and reduced errors in missing species due to high densities, along with ensuring quality control by preventing taxonomist eye fatigue.

A simple counting chamber consisted of three parts: a bottom part, the top of the chamber, and a cover glass. After settling was complete, the top portion of the chamber was slid off the bottom and a second cover glass was slid into place over the bottom chamber, which contained all the phytoplankton that settled. Chambers were cleaned with soap, water, and finally alcohol before reuse to remove residue from previous samples.

Samples were enumerated using phase-contrast illumination on a Leica DM IRB inverted microscope at 10 to 1000X magnification. Cells >15 µm were identified and counted using the 10X objective on transects that cover 50% of the chamber surface. This technique also ensured that all rare (in occurrence) taxa were identified and recorded. Cells <15 µm were counted on a single transect, 200 µm wide, at the center of the counting chamber using the 40X – 63X objective.

Alternatively, cells were also counted on 50 random fields of view on the counting chamber. This consisted of viewing the top, middle, and bottom of the chamber, while being sure not to identify and enumerate cells located along the edge of the chamber. Cells had to appear viable (i.e. chloroplasts intact). Cell fragments were not counted. Viable cells that were partially in the counting field on the right hand side were counted, but those on the left were omitted. For colonies, a small portion of the colony was counted and the number of cells then estimated. Cells within filaments were counted individually.

Typically a minimum of 400-600 cells should be enumerated to assure that the count was representative of the sample. All viable cells detected in each of the 50 fields were enumerated, which resulted in cell numbers typically > 1000. Estimates of cell volume for each species were obtained by routine measurements of 30-50 cells of an individual species and application of the geometric formula best fitted to the shape of the cell (Hillebrand et al 1999, Rott 1981, Vollenweider 1968). A specific gravity of 1 was assumed for cellular biomass. Cell counts were converted to wet-weight biomass by approximating cell volume at a later date, if required. Taxa were identified to species when possible using Desikachary (1959), Findlay and Kling (1979), Prescott (1982), and Komárek and Anagnostidis (1999; 2005).

The limit of quantification (LOQ) of cell counting was 1 cell/mL.

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1.1.5.2 MCYST Tests

Three MCYST methods were performed by the Alberta Centre for Toxicology, University of Calgary, Calgary, Alberta, Canada.

Treatment of water samples for PPI, ELISA and LC-MS/MS assays

For total MCYST analysis, water samples were frozen-thawed 3 times and later sonicated at full power (120 watt) on ice for 3 x 30 sec to disrupt the cyanobacterial cells. The samples were aliquoted for analysis by PPI, ELISA and LC-MS/MS assays. All samples were stored at -20°C until the analysis.

PPI

PPI tests were conducted in 96-well microplates. Samples were analyzed in 15 samples per batch on each 96-well microtitre plate.

For quality control and ensuring consistency between assays, negative and positive controls, a group of MC-LR standards, and quality control (QC) samples were run on each plate, in addition to the 12 water samples being tested. Negative control wells (n = 4) were used to determine baseline absorbance for all other wells on the plate. The positive control wells (n = 5) were used to quantify maximum enzyme activity under non-inhibitory conditions. The standards wells (n = 12) were diluted to final MC-LR concentration of 0.05, 0.10, 0.21, 0.34, 0.48, or 0.62 μg/L. QC samples included known concentrations of MCYSTs in lake water and Abraxis check samples; A, B, or C (tested each/plate).

Water samples were divided into sample blanks and sample test wells. The sample blanks wells (n = 2) allowed for normalizing absorbance between different water samples. The mean response of sample wells (n=3) was used to estimate the total MCYST concentration based on MC-LR (standards) concentration equivalent response.

Reagents were added to the wells, mixed well by stirring, and incubated at 37 °C in the dark. The absorbance for each well was measured at 405 nm using a spectrophotometer starting at 2 hours after incubation and after every 30 minutes until the absorbance of positive control wells was higher than negative control wells (the difference fell in the range of 0.45 to 0.6). At this point, the reaction was considered complete (generally after 3 hours).
Intra-plate (well to well) variation was monitored by %CV, which had to be <5% between replicates in each group. Inter-plate variation was monitored using two QC samples of known concentration. Results for the QC lake water samples had

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to be within mean ± 2SD or about mean ± 15% for all previous accepted plates (0.30 ± 0.045 µg/L). Abraxis check samples had to be within the commercial ranges (Check A, B, C: 0, 2 ± 0.5, and 20 ± 5 µg/L, respectively).

Sample concentrations were calculated by extrapolating from the standard curve. Samples above the linear range (>0.5 µg/L) were diluted and re-tested to provide an accurate result. Samples below the standard range were considered below the LOQ (<0.05 µg/L).

The quality of newly prepared standards and reagents were verified using current reagents. New reagents that produced results within ±15% of the target values were considered acceptable.

ELISA

The Microcystins-ADDA ELISA kit (Abraxis, Warminster, PA, USA) was used for ELISA testing. The test is an indirect competitive ELISA for the congener-independent detection of MCYSTs and nodularins. The test was conducted in microcystins-protein analogue-coated 96-well microplates. 40 samples were analyzed per 96-well microtitre plate. The intensity of yellow color formed at the final step of the assay as determined spectrophotometric ally, was inversely proportional to the concentration of MCYSTs present in the sample.

50µL of the standard solutions, control, and samples were added into the wells of the test strip. 50µL of the antibody solution was added to each well, mixed by moving the strip holder for 30 seconds, incubated for 90 minutes at room temperature, and then washed three times using the 1X wash buffer solution. 100µL of the enzyme conjugate solution was added to each well, mixed for 30 seconds, incubated for 30 minutes at room temperature, and washed three times. 100µL of (color) substrate solution was added to each wells, mixed, and incubated for 25 minutes at room temperature. Then 50µL of stop solution was added to the wells in the same sequence as for the substrate.

The absorbance at 450nm was determined spectrophotometrically using a microplate reader within 15 minutes after the addition of the stopping solution. The concentrations of the samples were determined using the standard curve included with each plate.

All standards, controls, and samples were analyzed in duplicate. Six commercial standards of graded concentrations (0, 0.15, 0.40, 1.0, 2.0, and 5.0 µg/L), provided by the manufacturer, were analyzed to prepare a standard curve of known toxin concentrations. A commercial control of known concentration (0.75 ± 0.185 µg/L) was provided with the kit as a test of standard curve accuracy. Abraxis check samples (check A, B and C) were used to verify the accuracy of assay in different ranges of MCYST concentrations (MC-LR = 0, 2 ± 0.5, and 20 ± 5 µg/L, respectively). Double-distilled water was used as a negative control to

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account for background noise. A series of calibrators (standards), negative controls (one 0 µg/L standard, one distilled water), a positive control sample and one of check sample were tested in every plate.

The tests were accepted if the average %CV of each entire plate was less than 10%, the positive control sample was within the range of 0.75 ± 0.185µg/L, and check samples were within the range of A: 0, B: 2 ± 0.5, and C: 20 ± 5 µg/L. The assay limit of detection (LOD) and LOQ were 0.1 and 0.15 µg/L, respectively. Linearity of the test was 0.15 – 5.0 µg/L. If the samples had concentrations over the linearity range, further sample dilution was performed.

LC-MS/MS

Chromatographic separation and mass spectrometric detection were performed using an Agilent 1100 LC and a Sciex API 4000 Triple Quadrupole Mass Spectrometer. For the LC method, 0.1% formic acid in D.I water and 0.1% formic acid in acetonitrile were used as mobile phase in gradient mode. The column (BDS Hypersil C18, 100x2.1mm, 5µ) was kept at 40 °C with a flow rate of 0.3 mL/min. The mass spectrometer was operated by ESI in positive mode. For MS/MS analysis, the identification and quantitation for each compound was performed based on its two MRM transition (in standard and unknown sample) combined with retention time. The MCYST levels were reported as the summation of the levels of MC-LR, MC-RR, MC-YR, MC-LW, and MC-LF in 2011/2012. In 2013, the levels of four additional variants, MC-LA, MC-LY, dMe-LR and MC-HtyR, were measured and added to the total MCYST levels.

A set of calibrators, an internal QC sample and an external QC were run with each batch of samples. The controls were run after calibrators, in the middle of the sequence and at the end of sequence. The controls were within ± 20% of the target values. The value on QC chart was reported. When the control was out of range, the entire batch was repeated. A chromatographic peak was considered acceptable when peak shape was symmetrical. The MRM ratios for the target compound and internal standard in the samples and control were within ± 20% of the ion ratios in the Calibrator.

Retention time (RT) and relative retention time (RRT) for the target compound in the calibrator, samples and control were within ± 3% of established values. RT for the internal standard in the calibrator, samples and control were within ± 3% of established values. When the autosampler failed in the middle of the run, samples that were not bracketed by controls were re-injected along with the Calibrators and Controls. The LOQ of LC-MS/MS for MC-LR, MC-RR, MC-LF, MC-LW, MC-LA and MC-LY is 0.1 µg/L, and the LOQ for MC-YR, dMe-LR and MC-HtyR is 0.2 µg/L.

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1.1.5.3 qPCR

qPCR test was performed by the Provincial Public Health Laboratory for Microbiology, Edmonton, Alberta, Canada.

Optimization of Sample Preparation for Amplification

Two sample preparation methods were used for the 20 reference samples to optimize sample volume for DNA extraction as follows:

1. a 2-ml aliquot of water was centrifuged at 8,000 rpm for 6 min and the supernatant collected. The pellet was dissolved in 200 µl TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0). The supernatant and pellet solution (200 µl/each) were used for DNA extraction; and
2. in order to simplify methods and reduce sample volume, 400 µl of water without any manipulation was directly used for DNA extraction. DNA yields from two different methods were quantified by qPCR assay.

After comparison of two methods, method 2 was adopted and applied in all remaining sample preparation for qPCR. DNA was extracted using a Qiagen DNA mini kit according to the manufacturer's instruction (QIAGEN Inc., Ontario, Canada). DNA was eluted with 50 µl elution buffer and stored at -20°C until further processing for qPCR.

qPCR for Detection of *mcy*E Gene

Primers and TaqMan probe targeting *mcy*E gene of *microcystis* spp were previously described by Sipari (Sipari et al 2010). The forward primer 5'-AAGCAAACTGCTCCCGGTATC-3' and the reverse primer 5'-CAATGGGAGCATAACGAGTCAA-3' were expected to yield a 120bp amplicon. TaqMan probe: 5'-CAATGGTTATCGAATTGACCCCGGAGAAAT-3' with a FAM 5' end label and a TAMARA 3' end label was used for real-time detection during the PCR reaction. 20 µl of the PCR reaction mixture containing 5 µl DNA solution, 0.5 µM of each primer, 0.125 µM probe, and 1 x LightCycler TaqMan Master Mix (Roche Diagnostics, Laval, Canada) was added to the capillaries (Roche Diagnostics). The capillaries were mounted onto the carousel, centrifuged and loaded into the LightCycler 1.0 instrument (Roche, Canada). The thermal cycles were as follows: an initial 10 min at 95°C, followed by 45 cycles of 10 sec denaturing at 95°C, 20 sec annealing at 58°C, and 1 sec extension at 72°C. Data analysis was automatically performed using the LightCycler software (version 4.0).

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Development of Standard Curve for Quantitation

A standard curve was established as a correlation between the *mcy*E gene copy numbers and the Ct (Koskenniemi et al 2007). A 529 bp fragment was amplified using primers designed from the same *mcy*E gene region covering the full length of targeting *mcy*E sequence. Primer sequences for the *mcy*E fragment were forward: 5'-AACCCGAAATGACTCAAGAAAAA-3', reverse: 5'-TCAAAAATACCGATAGGATGTT-3'. The fragment DNA was purified from the PCR product using a QIAquick PCR purification kit (QIAGEN Inc.) and quantified using NanoDrop 2000 spectrophotometer (Thermo Scientific, Canada). The molecular weight of fragment DNA was calculated. A series of 10-fold dilutions (2.0E+00 to 2.0E+09 copies) were analyzed by real-time PCR to identify the dynamic range and establish standard curve for quantitation of mcyE and 16S rRNA genes. The purified DNA was dispensed in aliquots containing 1.0E+03 or 1.0E+05 copies per 5 µL as positive control and stored at -70°C until use.

Development of an Internal Control for Monitoring PCR Inhibition

Salmon testes DNA (Sigma, Canada) was dissolved in water at a concentration of 1 mg/ml with stirring at room temperature for 2-4 hours. A qPCR for detection of salmon testes DNA was previously described (Haugland et al 2005). An appropriate amount of salmon DNA identified in Ct = 30 using real time PCR was used for monitoring inhibition of qPCR. Briefly, 5 µl of salmon DNA (Ct = 30) was added into a 400-µl water sample followed by DNA extraction and qPCR was performed for detection of salmon DNA. Inhibition was defined as a delay of Ct by 3 cycles as compared to a distilled water control spiked with the salmon DNA.

Quality Assurance and Quality Control (QA/QC)

Data analysis was automatically performed using the LightCycler software (version 4.0). The copy numbers of *mcy*E gene was obtained based on the standard curve. Data was entered into Excel and converted into final report number (unit: copy/mL).

A *microcystis mcyE* positive sample was aliquot in 500 µl/tube and frozen at -20°C, which was used in each extraction as positive control. Water was used in each extraction as negative control. Both controls were used to monitor each extraction process. Standard positive control (1.0×10^3 and 1.0×10^5 copies/5ul) and non-template control were included in the qPCR to monitor the PCR process. Salmon DNA was used as control to monitor the whole process. Negative controls including extraction control and non-template control must be truly negative in PCR reaction. Standard positive control had to fall in the accepted ct range.

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Accepted range for 1.0×10^3 (mean ± SD from 24 replicates) is 27.19 ± 0.22.

Accepted range for 1.0×10^5 (mean ± SD from 24 replicates) is 20.48 ± 0.23.

1.1.6 Method Validation

1.1.6.1 Data organization

The data of MCYSTs assays (PPI, ELISA, and LC-MS/MS) were merged with the data of qPCR and cell count using unique sample IDs. Data reported to have values under LOQ were treated as zero. Some assays were not performed on certain samples and these were taken as missing values. Only data from composite samples was used in the assessment and data from other types of water samples (i.e. grab samples) were excluded.

Since the distribution of data in PPI, ELISA, LC-MS/MS, qPCR, and total cell count were skewed, a logarithmic transformation was applied during data processing. In correlation analysis, the reported values under detection limits were excluded from the analysis.

1.1.6.2 Statistical Analysis

Linear regression was used to analyze the correlation among five lab test methods. Statistical significance was reported at α=0.05 level. Data processing and statistical analysis were carried out using Microsoft office EXCEL and ACCESS, SPSS and SigmaPlot.

1.1.6.3 Reproducibility

The laboratory methods are assessed by variation of analyses. The coefficient of variation (CV) were used for evaluating reproducibility. The formula is as following:

$$\%CV = SD \times 100 / X$$

where SD is the standard deviation, and X is mean. The acceptable %CV for three laboratory methods is less than 20%.

1.1.6.4 Accuracy

In the recovery experiment, a sample was first fortified or spiked with known amounts of specific MCYST. The levels of the MCYST were then measured

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using LC-MS/MS method. The accuracy of the method can be estimated by the term of recovery, which was calculated as measured value divided by the expected (assigned) value for the added (spiked) material. The equation of recovery was calculated as following:

$$\text{Recovery (\%)} = [\text{Reported result/spiked concentration}] \times 100$$

1.1.6.5 Limits of Detection

The Limit of Detection (LOD) is defined as the smallest amount or concentration of analyte that can be reliably measured. The Limit of Quantitation (LOQ) is the level of analyte above which quantitative results may be obtained with a specific degree of confidence. For the methods described in this report, the LOD and LOQ of ELISA were provided by the test kit. The LOD and LOQ of PPI and LC-MS/MS obtained from repeated experiments in which LOD and LOQ showed the same concentrations.

1.1.6.7 Matrix effects

Components other than the analyte of interest present in the sample could have an effect on measurement. Matrix effect (ME) was evaluated in LC-MS/MS method by comparing spiked MCYST concentrations in lake water and deionized water.

1.1.6.8 Reference materials

This forms an important part of QA/QC. Microcystin Check Samples (Abraxis) were used as external QC samples in PPI, ELISA, and LC-MS/MS assays. The internal QC for PPI assay was lake water from previous year with known MCYST concentration. ELISA positive internal QC samples were provided by the manufacturer of the ELISA kit, while spiked samples with MC-LR, MC-RR, MC-YR, MC-LF, and MC-LW were analyzed as internal QC for LC-MS/MS assay.

1.1.6.9 Sensitivity and specificity in comparison of methods

		Method 1 (Standard)		
		Positive	Negative	Total
Method 2	Positive	a (True Positive)	b (False Positive)	a + b
	Negative	c (False Negative)	d (True Negative)	c + d
Total		**a + c**	**b + d**	**a + b + c + d**

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1. Sensitivity measures the ability of Method 2 for detecting positive samples that are identified as positive by Method 1. Specificity measures the ability of Method 2 for identifying negative samples that are found to be negative by Method 1. Sensitivity and specificity are expressed as:

$$\text{Sensitivity} = a/(a+c)$$

$$\text{Specificity} = d/(b+d)$$

2. Positive predictive value (PPV) indicates the proportion of positive samples detected by Method 1 that are identified as positive by Method 2. Negative predictive value (NPV) indicates the proportion of negative samples detected by Method 1 that are identified as negative by Method 2. PPV and NPV are expressed as:

$$PPV = a/(a+b)$$

$$NPV = d/(c+d)$$

3. False positive rate (FPR) measures the probability of detecting a sample as positive by Method 2 when it is identified as negative by Method 1. False negative rate (FNR) measures the probability of detecting a negative sample by Method 2 that is identified as positive by Method 1. FPR and FNR are expressed as:

$$FPR = b/(b+d)$$

$$FNR = c/(a+c)$$

1.2 Fish Samples

1.2.1 Field Collection

All fish samples were collected by AESRD using gill-netting and angling between Aug – Oct, 2012. The two sampling periods encompass the highest expected water concentrations of MCYST based on past observations. Eleven lakes were selected for this study based on issuance of advisories against recreational water use due to blue-green algae blooms in 2012. An additional two lakes that have never had advisories issued served as controls. One lake that had an advisory in 2011, but not 2012, was also included. In total, 14 lakes were sampled. The sampling locations are shown in Figure 4.

Each sample was kept on ice, and then frozen flat before shipment. Samples were individually bagged and tagged with a label with a unique number. The

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samples were shipped to ACFT where the fish were dissected and the central pieces of muscle collected for MCYST analysis.

1.2.2 Sample Information

Seven different species of fish were caught. A total of 561 individual fish were obtained of which 357 muscle samples were analyzed (Table 2).

1.2.3 Laboratory Method

The method was developed by ACFT. Individual muscle samples were prepared using an acetonitrile extraction method as follows: 1.5 g of fresh fish muscle was homogenized using Precellys® 24 (3x15 sec at 6500 rpm) in 3.5 mL of solvent (acetonitrile:water prepared in a 9:1 v/v ratio) and then centrifuged at 5000 rpm for 15 min. 1.8 mL of the resulting supernatant was added to a commercial QuEChERS[1] dispersive SPEkit containing 50mg of primary secondary amine (PSA) functionalized silica, 50mg of octadecylsilane functionalized silica (C18EC), and 150mg of magnesium sulfate and shaken by hand for 1min.

1. The use of PSA remove organic acids, sugars, and fatty acids.
2. The use of C18EC remove hydrophobic constituents (such as lipids or other fats including cell membrane components).
3. The use of $MgSO_4$ is as a drying agent (desiccant).

[1] Quick, Easy, Cheap, Effective, Rugged, and Safe

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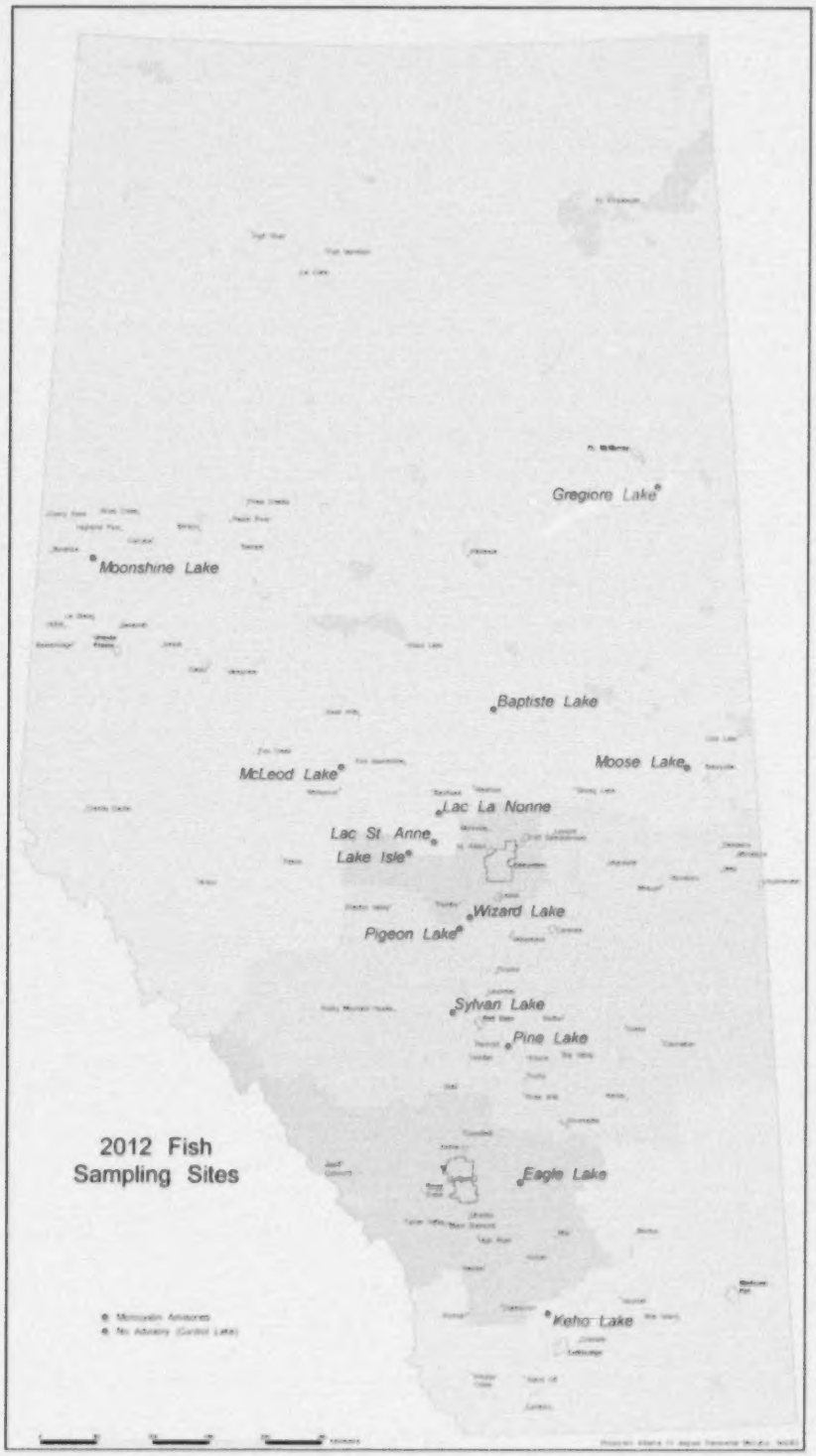

Figure 4 Fish Sampling Locations

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Table 2 Fish Sample Information

Water Body	Species	N	Weight (g)	Length (cm)	Tissue
Baptiste Lake	Northern Pike	8	995	57	Muscle
	Walleye	21	1066	50	Muscle
	Lake Whitefish	5 (dead)	908	40	Muscle
	Yellow Perch	1	37	14	Muscle
	Total	35			
Lac Ste Anne	Lake Whitefish	2	1055	46	Muscle
	Walleye	7	798	43	Muscle
	Total	9			
Eagle Lake	Northern Pike	11	755	49	Muscle
	Walleye	23	1323	48	Muscle
	Total	34			
Keho Lake	Lake Whitefish	4	1121	49	Muscle
	Northern Pike	30	1747	63	Muscle
	Walleye	24	1819	57	Muscle
	Total	58			
Lac la Nonne	Lake Whitefish	6	1642	52	Muscle
	Northern Pike	8	1028	55	Muscle
	Walleye	17	684	41	Muscle
	Yellow Perch	5	188	23	Muscle
	Total	36			
Lake Isle	Northern Pike	12	813	49	Muscle
McLeod Lake	Rainbow Trout	3	279	29	Muscle
Moonshine Lake	Rainbow Trout	6	321	30	Muscle
Moose Lake	Cisco	5	843	38	Muscle
	Lake Whitefish	3	2483	58	Muscle
	Northern Pike	13	1739	64	Muscle
	Walleye	38	1623	55	Muscle
	Yellow Perch	6	184	24	Muscle
	Total	65			
Pigeon Lake	Lake Whitefish	7 + 5 (dead)	1860	53	Muscle
	Northern Pike	3	1357	61	Muscle
	Walleye	31	1115	49	Muscle
	Total	46			
Pine Lake	Northern Pike	5	1418	58	Muscle
	Walleye	5	1112	48	Muscle
	Total	10			
Sylvan Lake	Lake Whitefish	3	702	41	Muscle
	Northern Pike	3	4600	87	Muscle
	Walleye	15	584	37	Muscle
	Total	21			
Wizard Lake	Northern Pike	15	841	50	Muscle
	Lake Whitefish	3	2110	52	Muscle
Gregiore Lake	Northern Pike	2	3425	76	Muscle
	Walleye	2	1225	49	Muscle
	Total	7			
Total		**357**			

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After the cleanup procedure, samples were again centrifuged for 15 min at 5000 rpm, and 0.5 mL of the resulting supernatant evaporated under nitrogen at 40 °C to dryness. The sample was then reconstituted in 0.5 mL of mobile phase and filtered using a 0.2 µm centrifugal filter prior to injection on LC-MS/MS.

Microcystins were separated on a Zorbax Eclipse XDB-C18 (100X2.1 mm, 3.5 µ) column using an Agilent 1100 HPLC and detected by a Sciex API 4000 Triple Quadruple Mass Spectrometer. Gradient mode was used to achieve the separation of analytes using mixtures of mobile phase A (0.1% formic acid) and mobile phase B (acetonitrile containing 0.1% formic acid) at a flow rate of 300 µl/ min. The injection volume was 20 µL. The linear gradient elution program started from 20% B and increased to 30% B in 2 min, 35% B in 5 min, 60% B in another 5 min, 98% of B in 6 min and held for 17 min. After each run, the column was equilibrated at initial conditions for 10 min before the next injection.

For the most sensitive quantitative analysis, the mass spectrometer was operated in the multiple reaction monitoring (MRM) mode, which included transitions of 520/ 135.2, 520/213.2 for MC-RR, 995.7/135.2, 995.7/213.2 for MC-LR, 1045.7/135.2, 1045.7/213.2 for MC-YR, 986.5/135.2, 986.5/213.2 for MC-LF and 1025.5/135.2, 1025.5/213.2 for MC-LW respectively. Nodularin was used as an internal standard. Dwelling time was set to 100 ms. The curtain gas was set at 12, collision-assisted dissociation (CAD) gas was at 8, Gas 1 was at 50, gas 2 was at 50, IS was at 5000 and the source heater probe temperature was at 500. Identification was based on retention time and the observed MRM ratio. Quantification is based on area ratio and a five point calibration curve (0, 5, 10, 20, 50 ng/g).

For quality control, a set of calibrators, a negative control, and one QC sample were prepared and run with each batch of samples. The QC samples were run after the calibrators and the end of the sequence. Two randomly selected samples spiked with 20 ng/g of MC-LR, RR, YR, LW and LF are prepared and run with each set of samples. The quality control must be within ±30% of the target values. The percent recovery for spiked samples must be between 70 to 130%. The calibration curve is acceptable if its correlation coefficient is ≥ 0.975. The maximum number of samples for each batch is 15.

1.2.4 Validation analysis

Reproducibility, recovery, matrix effect and method LOD & LOQ were evaluated.

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2. Results and Discussions

2.1 Beach Water Samples

2.1.1 qPCR

MCYST is produced by a MCYST synthetase enzyme complex encoded by the *mcy* gene cluster containing 10 genes (labelled *mcy* A to *mcy* J; Figure 5), and have been fully sequenced and characterized in species *Microcystis*, *Planktothrix* and *Anabaena* (Christiansen et al 2003, Nishizawa et al 2000, Rouhiainen et al 2004, Tillett et al 2000). The *mcy* genes have been used as molecular markers to detect MCYST-producing cyanobacteria (Rinta-Kanto et al 2005, Stotts et al 1993, Tillett et al 2001, Vaitomaa et al 2003). In this project, *mcy*E gene was selected as a target because it is responsible for the production of all known variants of MCYST (Harada et al 1999, Stotts et al 1993, Tillett et al 2000). Since *Microcystis* is one of the most common MCYST-producing species in Alberta lakes, the qPCR assay was developed for detection and quantitation of toxic *Microcystis sp.*

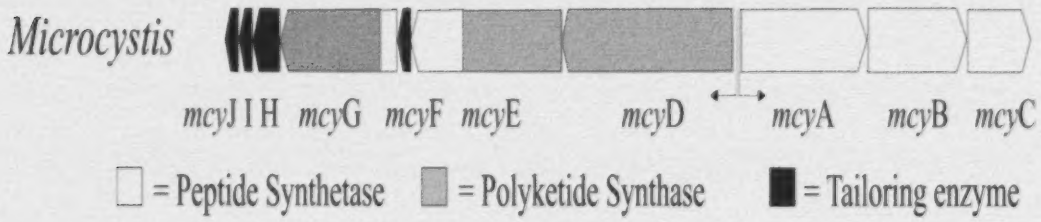

Figure 5 *mcy* Genes as Molecular Tools

The experimental sensitivity of qPCR assay was evaluated by performing the assay on samples with known *mcy*E levels. The *mcy*E gene quantitation was revealed in a linear log-range from 2.0 to 2.0 x 10^9 copies per reaction when the selected primers/probes were used in the qPCR reaction. The LOD was 50 copies/mL and the LOQ range was 500 to 5.0 x 10^{10} copies/mL for the assay. The qPCR efficiency was observed as 1.994 adjusting from the standard curve. The coefficient variation of Ct values from 24 replicates of qPCR was 1.12% for 1.0 x 10^5 and 0.79% for 1.0 x 10^3, respectively.

The experimental specificity of qPCR assay was evaluated by performing the assay on known toxin-producing and non-toxin-producing cyanobacteria species. It was evaluated using three M*icrocystis* strains including one non-toxic strain and two toxic strains (Table 3). No amplification of *mcy*E gene was observed in non-toxic CPCC124 with *mcy*E primers and probe targeting to *Microcystis*. 4.1 x 10^7 copies/mL of *mcy*E gene in CPCC299 and 9.78 x 10^5 copies/mL of *mcy*E gene in CPCC300 were detected, respectively.

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The qPCR assay developed has acceptable sensitivity and specificity. The quantitation of the assay is based on the standard curve, which is established as a correlation between the *mcy*E gene copy numbers and the threshold cycle (Ct) value (Figure 6).

Table 3 Specificity of qPCR for Detecting *Microcystis* Reference Strains

	CPCC124	CPCC299	CPCC300
*mcy*E (copy/mL)	negative	4.1×10^{7}	9.8×10^{5}
cell count (cells/mL)	2.97×10^{7}	2.14×10^{7}	4.2×10^{7}

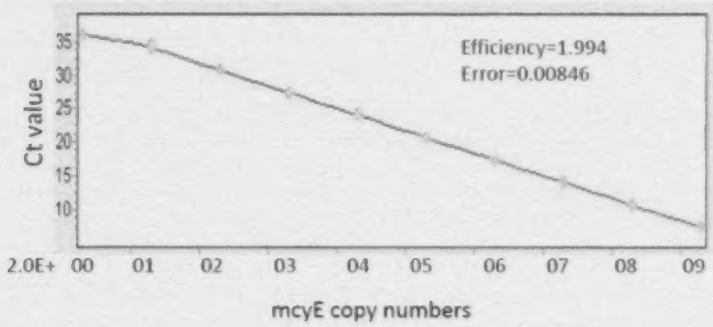

Figure 6 Threshold Cycle vs *mcy*E Copy for Quantification

The results from using two sample preparation methods - centrifuge supernatant/centrifuge pellet and direct extraction are illustrated in Figure 7. Since the two methods were consistent, the direct extraction method was selected for subsequent sample preparation.

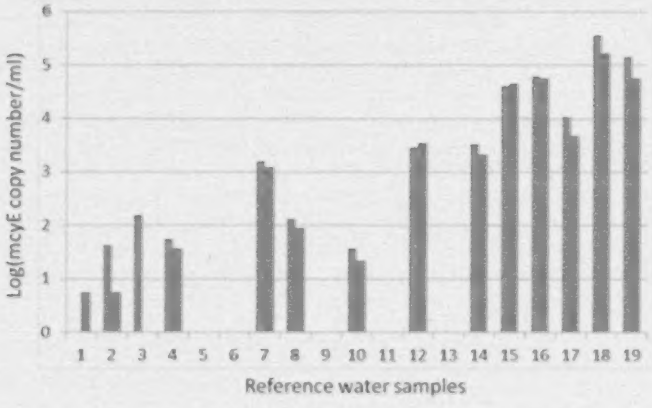

Figure 7 *mcyE* Copy Numbers in Water Samples in 2011

[Note: The sum of *mcy*E copy numbers from centrifuge supernatant and centrifuge pellet (blue) were compared with *mcy*E copy numbers from direct extraction (red) in 19 reference samples]

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2.1.2 MCYST Testing Methods

The acceptable criteria of QA/QC for PPI, ELISA, and LC-MS/MS assays are listed in Appendix B.

Method validation results of the PPI assay are summarized in Table 4. Intra- and inter-day reproducibility was assessed at two concentrations of 0.32 µg/L and 267 µg/L. The CVs for intra-day tests were 7% and 2%, respectively. The CVs for inter-day tests were 10% and 9%, respectively. The reproducibility for PPI is acceptable. The LOD and LOQ were 0.05 µg/L.

Table 4 Variation of PPI Assay

Concentration	CV%
Intra-day	
0.32 µg/L	7
267 µg/L	2
Inter-day	
0.32 µg/L	10
267 µg/L	9

Method validation results of the ELISA assay are summarized in Table 5. The intra- and inter-day reproducibility of ELISA was assessed by spiking water samples with MC-LR at the concentrations of 1, 5, 10, 15, 20, and 25 µg/L, respectively. The CVs for all concentrations were less than 20%. The LOD was 0.1 µg/L and the LOQ was 0.15 µg/L.

Table 5 Variation of ELISA Assay

Concentration	CV%
Intra-day	
1 µg/L	9
5 µg/L	13
10 µg/L	6
15 µg/L	13
20 µg/L	2
50 µg/L	6
Inter-day	
1 µg/L	14
5 µg/L	18
10 µg/L	5
15 µg/L	8
20 µg/L	16
50 µg/L	6

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Method validation results of the LC-MS/MS assay are summarized in Table 6. The CVs for intra- and inter-day tests of MC-LR, MC-RR, MC-YR, MC-LW, and MC-LF were less than 20%.

The recovery study was performed at 1 µg/L and 10 µg/L. The values recoveries MC-LR, MC-RR, MC-YR, MC-LW, and MC-LF were within the acceptable range of 80 to120 per cent.

Matrix study was performed at 1 µg/L and the matrix effect was within the acceptable range of 80 and 120 per cent for the 5 MCYST congeners. The LOD and LOQ for MC-LR, MC-RR, MC-LW, and LC-LF were 0.1 µg/L. The LOD and LOQ for MC-YR were 0.2 µg/L.

Table 6 Variation of LC-MS/MS Method for Water Samples

	CV%				
	LR	**RR**	**YR**	**LW**	**LF**
Intra-day	7	3	7	9	3
Inter-day	8	8	8	12	10
Recovery					
1 µg/L	96.6	90.7	90.8	100	99.5
10 µg/L	97.0	97.0	89.1	105	104
Matrix Effect			ME%		
1 µg/L	107	97	89	103	101

2.1.3 Comparison of Five Laboratory Methods

Five laboratory methods were used for 2012 and 2013 monitoring program. The principles and applications of the methods are summarized in Table 7.

Table 7 The Principles and Applications of the Methods

Method	Principle	Application
Visual Inspection	Observe surface scum in the field	Issuing advisories (AHS)
PPI	Quantify MCYST levels in water body (protein phosphatase inhibition)	Screening MCYST toxicity
ELISA	Quantify MCYST levels in water body (antibody reaction)	Screening MCYST toxicity
LC-MC/MC	Quantify MCYST levels in water body (chemical mass)	Confirming MCYST toxicity
Cell count	Identify and quantify cyanobacteria species in water bodies	Assessing bloom prevalence/ formation, identifying health hazards
qPCR	Quantify *mcy*E gene (live/dead cells, 2 wk cycle)	Developing early warning system

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A total of 1845 surface water samples were collected from 87 water bodies across Alberta from 2011 to 2013, and tested for MCYST using PPI; and 1170 of these samples were tested by LC-MS/MS (Table 8). Of the 1845, 636 samples from lakes with positive visual inspection were also tested by ELISA. Additionally, 1277 samples collected in 2012/2013 were tested by qPCR for the *mcy*E gene. Lastly, 630 samples were examined for algal species abundance by direct light microscopy and Automated FlowCam.

Table 8 Summary of Samples Size for Laboratory Analysis

	Cell Counting (microscopy)	mcyE by qPCR	MC-LR eq by PPI	MC-LR eq by ELISA	MC-LR eq by LC-MS/MS
Sample tested	-	1277*	1245**	-	1170**
Detected samples	-	58%	91%	-	41%
Sample tested	630*	-	636	636	636 ***
Detected samples	63%	-	90%	78%	62%

Note: 1. LOQ = Limit of Quantitation (i.e. lowest quantifiable MCYST concentration); 2. if observing 1 cell in one ml sample, report as detected; 3. in molecular diagnostic field, qPCR has a linear range from 125 to 2,500,000,000 copies. *All lakes in 2012/2013, **All lakes in 2011/2012/2013.*** Selected samples tested using ELISA.

Correlations

The correlations for five test methods are summarized in Table 9. The MCYST levels were correlated well among three assays, i.e., ELISA, PPI, and LC-MS/MS (r: 0.91– 0.96, $p < 0.001$). The findings are consistent with those in other studies ($r > 0.9$) (Babica et al 2006, Fischer et al 2001, Metcalf et al 2001, Ward et al 1997).

Table 9 Correlations of Five Laboratory Methods

	Sample Size	r	r^2	p-value
LC-MS/MS vs. PPI	484	0.91	0.83	<0.001
LC-MS/MS vs. ELISA	385	0.91	0.82	<0.001
ELISA vs. PPI	474	0.96	0.91	<0.001
qPCR vs. PPI	708	0.65	0.42	<0.001
qPCR vs. ELISA	336	0.59	0.35	<0.001
qPCR vs. LC-MS/MS	352	0.50	0.25	<0.001
Cell count vs. PPI	354	0.49	0.24	<0.001
Cell count vs. ELISA	230	0.41	0.17	<0.001
Cell count vs. LC-MS/MS	193	0.35	0.12	<0.001
Cell count vs. qPCR	275	0.34	0.12	<0.001

Note: 1. samples with "non-detected" levels of MCYST were excluded from analysis; 2. log transformation (natural base) for linear regression

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Although good correlation was observed, no one single method should be used for monitoring MCYST alone because they are based on different principles.

The PPI assay measures inhibition of enzymatic activity of protein phosphatases (either PP1c or PP2a - the target enzymes responsible for MCYST toxicity) by MCYST to estimate the total concentrations of toxin in water samples (An & Carmichael 1994, MacKintosh et al 1990). Because sample toxicity is extrapolated from a standards curve of pure MC-LR, total toxicity is usually expressed as MCYST-LR equivalents. The assay cannot discriminate between MCYST variants, nodularins or other compounds (i.e. tautomycin) capable of inhibiting activities of specific (type PP1c & PP2a) protein phosphatases. The fact that nodularin is produced primarily by *Nodularia*, a cyanobacteria inhabiting estuarine and marine environments means the likelihood of this toxin causing overestimation of MCYSTs in lake water samples in Alberta is remote. More likely is the possibility that tautomycin produced by native soil Actinobacteria could enter surface water during severe erosional run-off events (flooding), causing overestimation of MCYST via PPI assay. However, concentrations in soil are low making typical levels in surface water negligible. And given a much greater binding affinity of tautomycin for PP2A (vs PP1c), the use of PP1c in the assay can minimize the likelihood of overestimation of MCYST and false positives.

ELISA measures MCYST levels through highly specific interactions between an entity of the MCYST peptide structure and an antibody. Like PPI, the Abraxis ELISA cannot distinguish individual MCYSTs, but rather estimates the total MCYST level by quantifying antibody binding to the unique β-amino acid (ADDA) moiety of MCYSTs (Fischer et al 2001). Again, like PPI, because sample toxicity is extrapolated from a standards curve of pure MC-LR, total toxicity is often expressed as MC-LR equivalents. It is important to consider the degree of binding affinity for the antibody is not equal amongst MCYST congeners, nor is it a function of its toxicity. Some weakly toxic analogues (e.g. MC-RR) can have greater antibody binding affinity than more toxic MCYSTs. In addition, some non-toxic congeners may elicit a binding response in the assay. Thus, toxin estimation with ELISA is highly dependent on the MCYSTs contained within a sample and there is a tendency for overestimation of true toxin concentrations and likelihood of false-positives.

LC-MS/MS quantifies MCYST variants by separating and identifying individual MCYST. The total MCYST (∑MCYST) levels measured in this study were the summation of the levels of MC-LR, MC-RR, MC-YR, MC-LW, and MC-LF in 2011/2012 and the summation of the levels of MC-LR, MC-RR, MC-YR, MC-LW, MC-LF, MC-LA, MC-LY, dMe-LR and MC-HtyR in 2013. Expect for MC-HtyR, these eight variants are readily available in pure (commercial standard) form. As a result, it is important to note that reported concentrations may not represent the true sum of all possible known MCYST variants that could be present in a water

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sample. Depending on the actual MCYSTs contained in a sample, sum total concentration may underestimate true toxin levels if unknown (other than those tested variants) analogues are present and in rare instances, there is a likelihood of false negatives. However, the variants measured particularly MCLR are typically present at higher concentrations than the more obscure variants.

PPI and ELISA are often used for screening (estimating) and LC-MC/MC is often used for confirmation.

The MCYST levels measured by these three assays and *mcy*E copies detected by qPCR were also correlated, but at a lesser extent (r: 0.50 – 0.65, $p < 0.001$). qPCR was used to detect the genomes of toxin-producing *Microcystis* species that encode for enzymes (e.g., *mcy*E) that are involved in the biosyntheses of these hepatotoxins. It should be noted that other cyanobacteria – primarily *Anabaena* and *Planktothrix* spp – common in Alberta's surface waters, also possess specific *mcy*E genes. Failure to include quantification of these in water samples could severely underestimate the capacity for MCYST production. Also, moderate correlations are likely due in part to some cyanobacteria species or strains not fully expressing the *mcy*E genes to produce MCYST.

MCYST biosynthesis is a multi-step process. The *mcy*E gene is involved in one of these steps (Dittmann et al 2013). MCYST biosynthesis is also influenced by life stage and many environment factors. For example, iron depletion in water may increase MCYST levels (Alexova et al 2011).

The cell density of cyanophyceae was fairly correlated with the MCYST levels measured by PPI and ELISA (r: 0.49-0.41, $p<0.001$), but not with LC-MS/MS and qPCR. Cell counting is a direct and sensitive method for the detection of cyanobacteria in the water samples. As the results present in Appendix C, some MCYST-producing species were observed in most lakes under public health advisories, but cell density varied from one lake to another. It may explain this moderate correlation result.

The inconsistent relationship between cell counting and toxicity testing/qPCR may result from (1) MCYST being released into the environment from dying or dead cyanobacteria that were not counted, (2) *mcy*E present only in toxin-producing strains of several genera of cyanobacteria, and (3) the influence of external (non-genetic) factors on the gene expression and concomitant toxin production is often regulated (Qian et al 2012, Ray & Bagchi 2001, Sivonen 1990, Wiedner et al 2003).

Sensitivity and Specificity

Five MCYST analogues of MC-LR, MC-RR, MC-YR, MC-LW, and MC-LF were analyzed for 1170 samples collected in 2011, 2012 and 2013. ∑MCYST levels

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were detected in 41 per cent of the samples. MC-LR congener accounted for 0 to 100 per cent of ∑MCYST congeners.

The sensitivity and specificity of PPI and ELISA were evaluated as compared to (∑MCYST) analyzed by LC-MS/MS according to the cut-off values of 20 µg/L of recreational water guideline and 1.5 µg/L of drinking water guideline, respectively. The results are showed in Table 10. The detailed information is present in Appendix D.

Table 10 Sensitivity and Specificity for Toxicity Testing

Compared To LC/MS/MS	Sensitivity	Specificity	PPV	NPV	FPR	FNR
20 µg/L guideline						
PPI (%)	90	99.5	81.3	99.7	0.5	10
ELISA (%)	100	95.2	47.3	100	4.8	0
1.5 µg/L guideline						
PPI (%)	96.6	95.3	78.3	99.4	4.7	3.4
ELISA (%)	100	77.2	58.9	100	22.8	0

PPV = Positive predictive value, NPV = Negative predictive value, FPR = False positive Rate, FNR = False negative rate

As compared to LC-MS/MS, PPI assay had 90 per cent and 96.6 per cent of sensitivity, 99.5 per cent and 95.3 per cent of specificity, and 10 per cent and 3.4 per cent of the false negative rates using 20 µg/L and 1.5 µg/L cut-off values, respectively. When the 20 µg/L cut-off was used, 3 samples showed false negative among 29 samples. When the 1.5 µg/L cut-off was used, 6 samples showed false negative among 176 samples. The false positive rates of 0.5 per cent (20 µg/L cut-off point) and 4.7 per cent (1.5 µg/L cut-off point) were observed for PPI assay.

False negative results from using PPI assay have been previously reported (Sim & Mudge 1993, Ward et al 1997). False negative result may arise due to the following reasons: (1) the estimated MC-LR equivalent concentrations (via PPI assay) could be lower if some MCYST variants in water samples have lower toxicity than that of MC-LR (An & Carmichael 1994), and (2) contamination during methanolic extraction may mask the presence of cyanotoxin in water samples (Ward et al 1997).

As an example, samples containing high levels of MC-RR, an analogue that is significantly (≈1000x) less-toxic (based on i.p injection in mice) compared to MC-LR, would yield a lower concentration via PPI assay than by LC-MS/MS (specific quantification). It is also important to note when considering the two cut-off values in this report, a false negative does not indicate a complete failure to

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detect toxin, it only indicates a failure to detect toxin at a level equal to or greater than the specified cut-off level.

As compared to LC-MC/MC, ELISA assay showed 100 per cent of sensitivity and 95.2 per cent of specificity using 20 µg/L as cut-off and 100 per cent of sensitivity and 77.2 per cent of specificity using 1.5 µg/L as cut-off. The false negative rates were 0 per cent. The false positive rates of 4.8 per cent and 22.8 per cent were observed as using 20 µg/L and 1.5 µg/L values, respectively.

Overestimation of ∑MCYST concentrations using the Abraxis kit may result from the binding of other ADDA-containing molecules (e.g., nodularin), but as mentioned above, this either occurs in cyanobacteria native to estuarine and marine environments or can be caused by degraded MCYST (Fischer et al 2001, Hawkins et al 2005).

Most likely, the overestimation of MCYST by ELISA stems from a combination of: 1) quantification of unknown MCYSTs (those analogues in the sample not accounted for by the limited LC-MS/MS standard suite); 2) degraded MCYST fragments; and 3) the unequal and unusually high binding affinity some analogues have for the antibody compared to others.

Continuing with the example above, MC-RR is significantly less-toxic (based on i.p injection in mice) than most analogues due to its higher water solubility. However, it possesses greater binding affinity with the ELISA antibody than other, more toxic congeners. Thus, samples containing high levels of MC-RR would easily be overestimated by ELISA compared to actual concentrations.

This (usual overestimation) also explains why ELISA demonstrates 100% sensitivity and a high false positive rate compared with LC-MS/MS. Again, comparing total toxin concentrations of the same sample may yield lower values with PPI assay. Antibody affinity has no relationship to toxicity, making comparisons between ELISA and PPI somewhat contentious.

2.1.4 Cost-effectiveness

The estimated cost and time for four laboratory methods are summarized in Table 11. The estimated costs were related to materials and time for running the samples. Labour and equipment were not included. ELISA has the highest average cost at $40 per sample, PPI and cell counts by FlowCam have the lowest average cost at $5 per sample. The lengths of time required for running a sample are 0.25 hour for cell counts, 0.5 hour for LC-MS/MS, 1.5 hours for qPCR, and 6 hours for PPI and ELISA.

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LC-MS/MS cannot be operated in parallel mode to handle several samples at the same time, whereas qPCR, PPI, and ELISA can process multiple samples during one run. Therefore, the latter methods significantly cut down the experimental time required per sample.

Table 11 Material Cost and Time for Four Laboratory Methods

	Cost per sample	Time per test (hr)	Batch sample (N)	Time per batch-test (hr)	Time per sample as batch-test
Cell counts (FlowCam)	$5	0.25	10	2.5	15 min
qPCR	$15	1.5	24	3.5	9 min
PPI	$10	6	28	14	30 min
ELISA	$40	6	90	22	15 min
LC-MS/MS	$10	0.5	30	23	46 min

2.2 Fish Samples

Method validation results for detecting MCYST in fish samples using LC-MS/MS are summarized in Table 12. Uncontaminated fish muscle fortified with MCYST mixture was extracted to validate the method. MCYST were linear up to 200 ng/g ww with correlation coefficient greater than 0.98. Reproducibility was performed at 10 ng/g, 50 ng/g and 100 ng/g. The CVs were below 12 per cent. The recoveries for MC-LR, MC-RR, MC-YR, MC-LW, and MC-LF were 80, 88, 91, 83, and 79 per cent, respectively. The values of recoveries for these five MCYST variants were within the acceptable range of 80 to 120 per cent. A matrix study was performed at 50 ng/g and the matrix effect for MC-LR, MC-RR, and MC-YR were 106, 127, and 117 per cent, respectively. The LOD and LOQ for MC-LR, MC-YR, MC-LW, and MC-LF in fish muscle were 5 ng/g ww. The LOD and LOQ for MC-RR in fish muscle were 5 ng/g ww and 10 ng/g ww, respectively.

Table 12 Variation of LC-MS/MS Method for Fish Muscle Samples

	LR	RR	YR	LW	LF
CV (%) at 10 ng/g (MCYST)	11	9.6	12.2	9.1	11
CV (%) at 50 ng/g (MCYST)	2.3	7.3	5.7	7.0	2.3
CV (%) at 100 ng/g (MCYST)	5.2	9.6	5.4	10	5.2
Recovery (%)	80	88	91	83	79
CV (%)	3.5	7.9	5.4	7.6	6.0
Matrix Effect at 50 ng/g (ME%)	106	127	117		
LOD (ng/g ww)	5	5	5	5	5
LOQ (ng/g ww)	5	10	5	5	5

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3. Conclusions

All five laboratory methods are valid and acceptable for determining cell density and MCYST levels in recreational water and fish tissues in terms of QA/QC standards, reproducibility, reliability, sensitivity and specificity.

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Part II

Characterization of Cyanobacteria and Microcystins in Alberta Beach Water

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Each summer, many Alberta lakes that are popular destinations for recreational activities experience cyanobacterial blooms. Since 2010, AHS has been monitoring cyanobacteria blooms in Alberta' lakes and reservoirs with public recreational beaches. AHS developed management procedures to issue public health advisories each year. In 2010 and 2011, the trained environmental health officers and students from the environmental health program conducted visual inspections to judge the severity of cyanobacteria blooms. Surface water samples were collected adjacent to beach areas and sent to the Alberta Centre for Toxicology for MCYST analysis by using PPI method.

In 2012 and 2013, AH worked closely with AHS, AESRD, public health laboratories and academic researchers to conduct a comprehensive monitoring program. The objectives of Part II are to:

1. determine cyanobacteria density and species in selected recreational water samples,
2. determine MCYST levels in all targeted water samples,
3. determine *mcy*E gene copies in selected water samples,
4. demonstrate spatial distribution of MCYST levels, and
5. explore time trends of cyanobacteria density and MCYST levels.

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1 Methods and Materials

Sampling and Laboratory Analysis

Information on sampling and laboratory analysis is described in the Part I.

GIS Mapping Methods

All lakes/reservoirs were mapped and sampling sites and characteristics of each sample indicated.

Sampling date is indicated for each point and a number placed beside the circle indicates the number of samples at a site for those instances when several samples were collected within the same time period.

Sampling sites were usually beaches. In most cases the name of the beach and circles denoting beach location and date of collection is indicated. Circles are placed in chronological order within a beach location (circles do not denote exact sampling site) in those instances where a beach name is not indicated (in lakes with a single beach), the location of a single beach was used to indicate sample locations.

Maps were made for the different types of analysis performed, these include:

1. Total cyanophyceae (Cell Counts per mL). These were highlighted with three colors:

    a. White = observed value of 0
    b. Green = observed value of <100,000
    c. Red = observed value ≥100,000

2. *mcy*E by qPCR (Copy/mL). There are no standards *myc*E and examining the patterns of above/below guidelines with other tests since there were consistent results across the different types of analysis to create the categories. The categories were established based on statistical analysis of the results. These were:

    a. White = *mcy*E Detection
    b. Green = observed value of <60,000
    c. Red = observed ≥ 60,000

3. Microcystin Equivalent by PPI levels (µg/L):

    a. Green = observed value < 20

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b. Red = observed value of ≥ 20

4. Microcystin Levels (µg/L). Four categories were used:

   a. Dark Green for an observed value <1.0 (WHO Water Guideline)
   b. Light Green for an observed value <1.5 (Canadian Drinking Water Guideline)
   c. Orange for an observed value of <20 (WHO Recreational Guideline)
   d. Red for an observed value of ≥ 20

All maps were created using Canvas+GIS v14. Cartographica v1.4.2 was used to create the GIS files from spreadsheet information.

Statistical Analysis

Linear regression was used to analyze the correlation among five lab test methods. Statistical significance was reported at α=0.05 level. Data processing and statistical analysis were carried out using Microsoft office EXCEL and ACCESS, SPSS and SigmaPlot.

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2 Results and Discussions

2.1 Beach Water

2.1.1 MCYST Concentrations

MCYST concentrations in water samples collected between 2010 and 2013 are summarized in Table 13. The sample information is listed in Appendix E and F.

Table 13 MCYST Concentrations in Water Samples

Year	2010	2011	2012	2013
Lakes	39	33	47	49
Total Sample#	544	459	573	612
PPI				
Total Sample #	544	459	569	608
≥ 0.05 µg/L*	502	434	549	509
% of detected	92	95	96	84
≥ 20 µg/L**	7	13	9	10
% of detected	1	3	2	2
Geo mean (µg/L)	0.2	0.3	0.3	0.2
Mean (µg/L)	1.1	2.0	1.8	1.0
Range (µg/L)	nd – 35	nd – 78	nd – 100	nd – 64
SD	3.81	8.28	7.0	4.8
ELISA				
Total Sample#	-	83	262	261
≥ 0.15 µg/L*	-	68	229	170
% of detected		80	87	65
≥ 20 µg/L**	-	18	23	12
% of detected		22	9	5
Geo mean (µg/L)	-	5.8	2.1	1.0
Mean (µg/L)	-	16	7.1	3.8
Range (µg/L)	-	nd – 165	nd – 342	nd – 198
SD		29.7	25.2	16.2
LC-MS/MS				
Total Sample#	-	83	562	315
≥ 0.1 µg/L*	-	59	224	134
% of detected		71	40	43
≥ 20 µg/L**	-	13	8	1 8
		16	1	3
Geo mean (µg/L)	-	3.6	1.1	0.8
Mean (µg/L)	-	7.3	1.7	2.7
Range (µg/L)	-	nd – 62	nd – 173	nd – 174
SD	-	13.8	9.5	15.0

*LOQ , **Guideline value; nd: not detected

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The PPI assay was used to determine MCYST levels for all beach water samples collected from 2010 to 2013. Selected samples collected in 2011/2013 and all samples collected in 2012 were tested by ELISA and LC/MS/MS.

Levels of MCYST determined by using PPI analysis for 2010 and 2011 are illustrated in Figures 8 and 9, respectively. Alberta beach monitoring locations for 2012 and 2013 are illustrated in Figure 10 and 11, respectively. Lakes were marked in red color as "Advisory Lakes" either by visual inspection of cyanobacterial bloom or by laboratory confirmation of cell density exceeding recreational guideline levels. The detailed sample distribution maps by cell counting are illustrated in Appendix C.

The numbers of lakes with public beach access monitored in 2010, 2011, 2012 and 2013 were 39, 33, 47 and 49, respectively. The majority lakes with MCYST levels exceeding 20 µg/L were located in central and Northern Alberta. In Southern Alberta, MCYST levels exceeding 20 µg/L frequently occurred in Eagle Lake.

In 2010, MCYST exceeded 20 µg/L in Isle Lake, Lac La Nonne, Lac Ste Anne, Pine Lake and Thunder Lake. Of 544 samples collected, MCYST was detected in 92 per cent MCYST exceeded 20 µg/L in 1 per cent of these. The geometric mean MCYST concentration was 0.2 µg/L.

In 2011, MCYST exceeded 20 µg/l in Baptiste Lake, Eagle Lake, Isle Lake, Pigeon Lake, Thunder Lake, and Wizard Lake. Of 459 samples collected, MCYST was detected in 95 per cent (by PPI assay). In addition, ELISA and LC-MS/MS were conducted for 83 samples which were selected from the samples with relatively high MCYST levels by using PPI. MCYST exceeded the recreational guideline (20 µg/L) in 3 per cent samples analyzed by PPI, 22 per cent by ELISA, and 16 per cent by LC-MS/MS. The geometric mean MCYST concentration was 0.3 µg/L, 5.8 µg/L, and 3.6 µg/L by PPI, ELISA, LC-MS/MS, respectively. MCYST measured by ELISA and LC-MS/MS in 2011 tended to be higher than those measured in 2012 and 2013.

In 2012, MCYST exceeded 20 µg/L in Baptiste Lake, Battle Lake, Calling Lake, Cross Lake, Eagle Lake, Gregoire Lake, Isle Lake, Kehewin Lake, Lac La Nonne, Lac Ste Anne, McLeod Lake, Moonshine Lake, Moose Lake, Pigeon Lake, Pine Lake, Skeleton Lake, Stoney Lake, Thunder Lake, and Vincent Lake.

Of 573 samples collected, MCYST was detected in 96 per cent (by PPI). MCYST exceeded 20 µg/L in 2 per cent samples by PPI, in 9 per cent by ELISA, and in 1 per cent by LC-MS/MS. The geometric mean MCYST concentration was 0.3 µg/L, 2.1 µg/L, and 1.1 µg/L by PPI, ELISA, LC-MS/MS, respectively.

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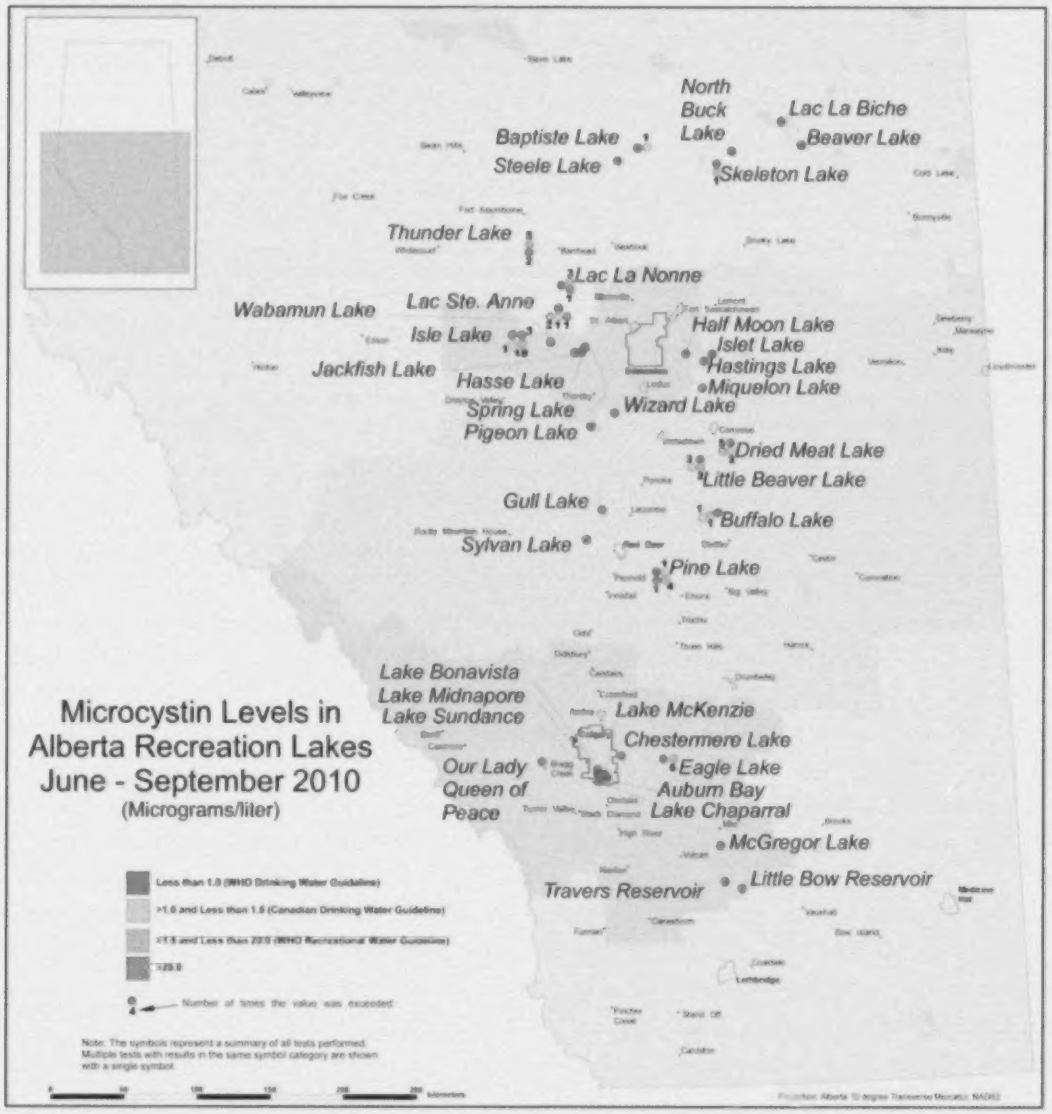

Figure 8 MCYST Levels in Beach Water in 2010

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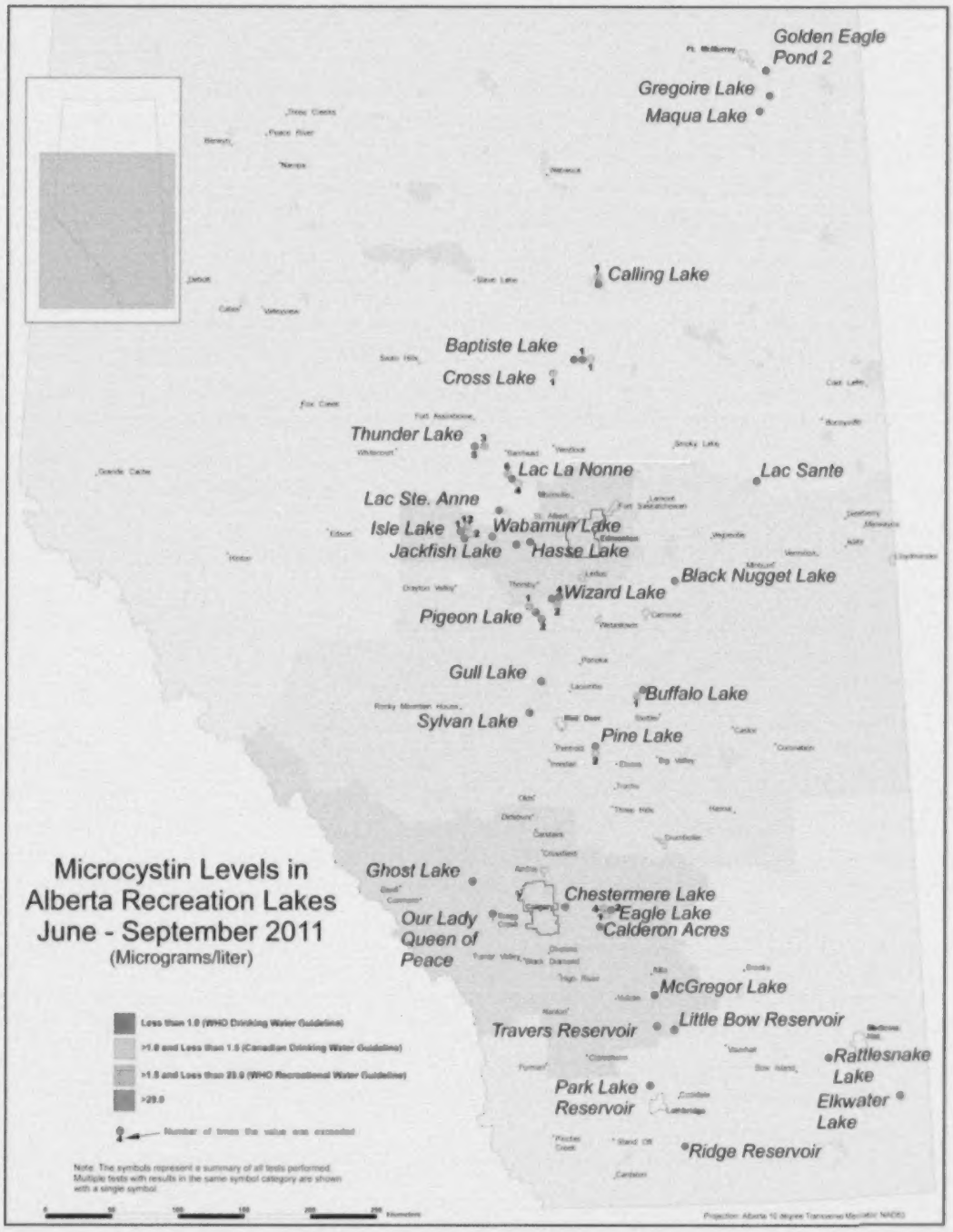

Figure 9 MCYST levels in Beach Water in 2011

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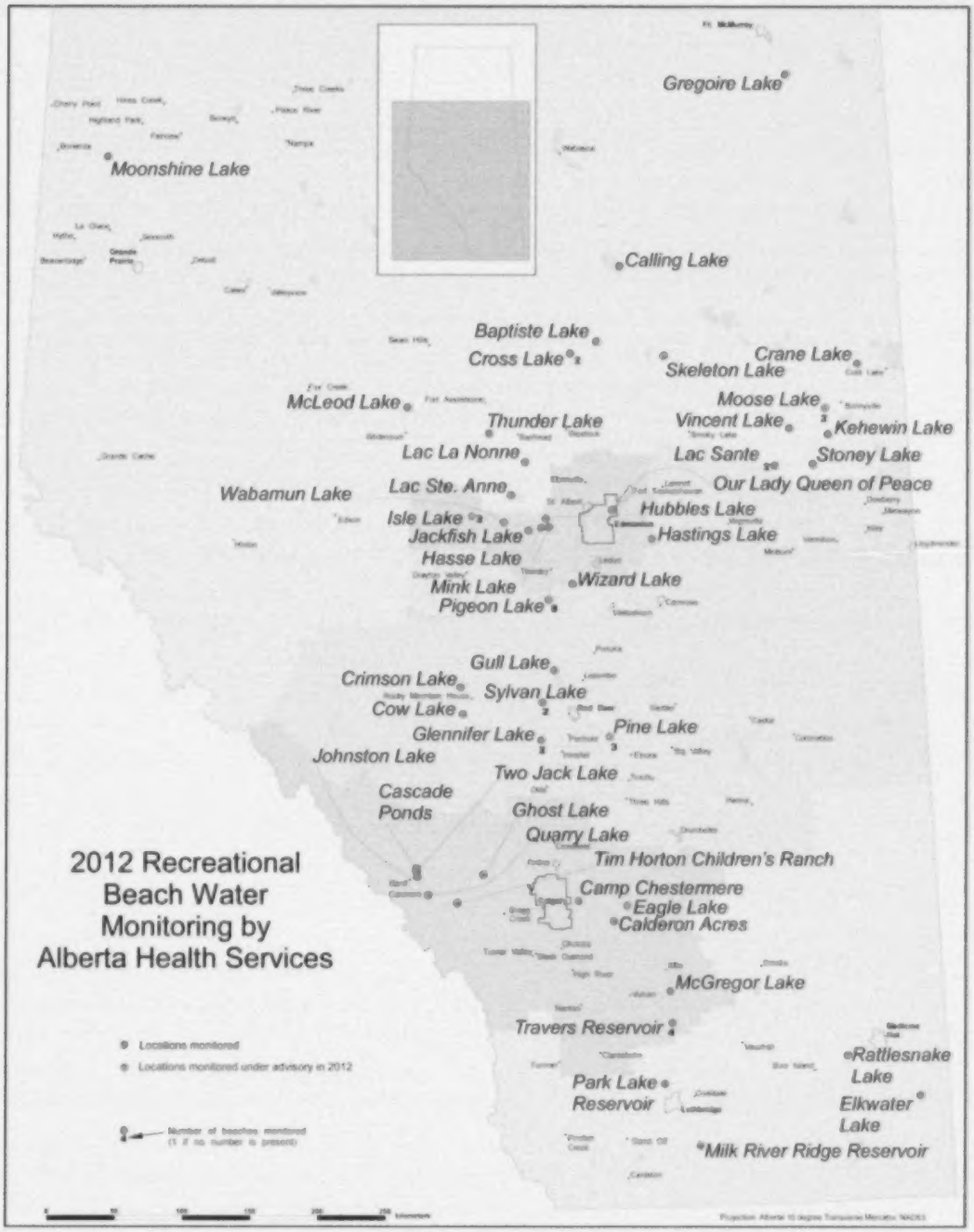

Figure 10 MCYST levels in Beach Water in 2012

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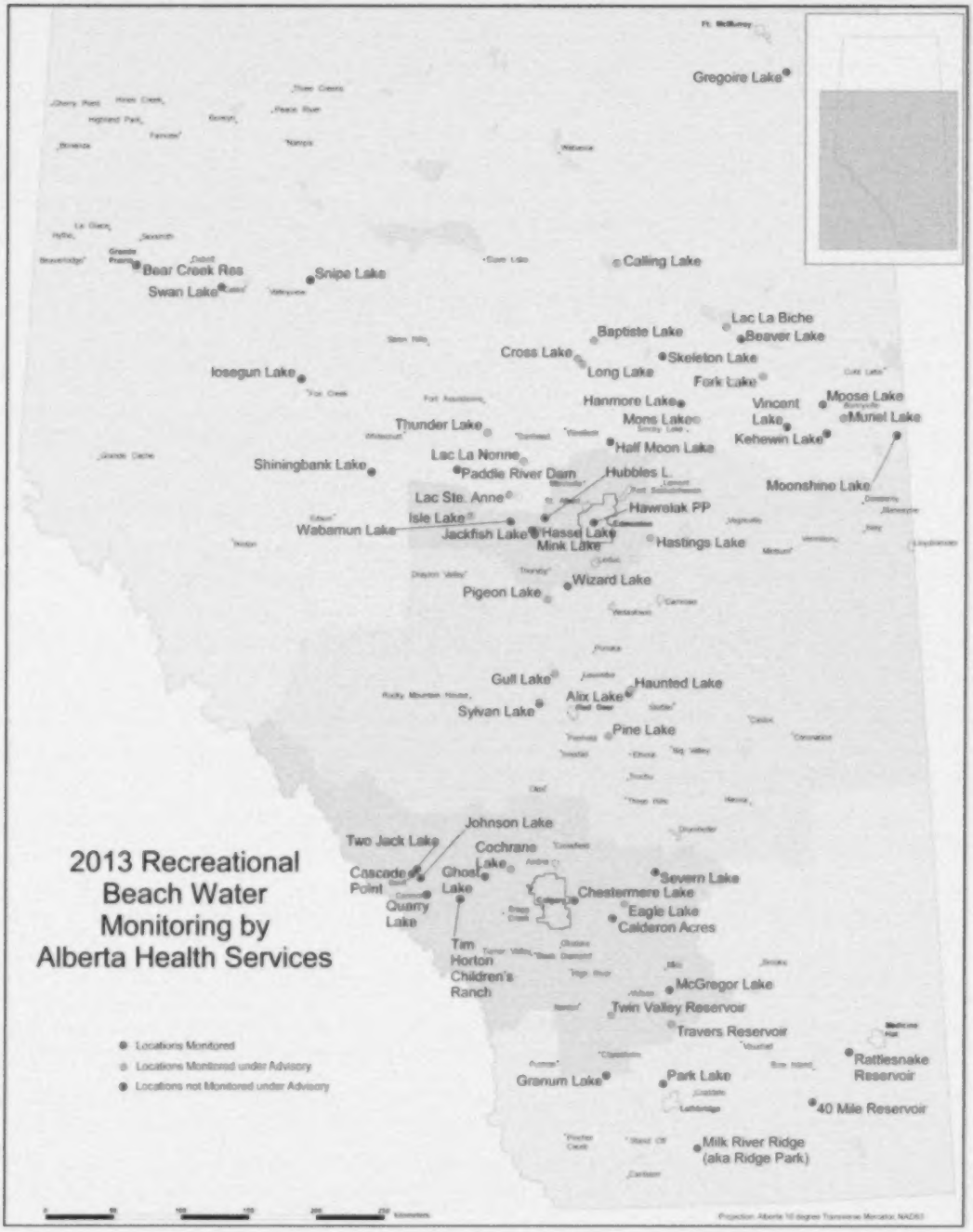

Figure 11 MCYST levels in Beach Water in 2013

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In 2013, MCYST exceeded 20 µg/L in Cochrane Lake, Eagle Lake, Haunted Lake, Isle Lake, Lac La Nonne, and Muriel Lake. Of 612 samples collected, MCYST was detected in 84 per cent (by PPI). MCYST exceeded 20 µg/L in 2 per cent samples by PPI, in 5 per cent by ELISA, and in 3 per cent by LC-MS/MS. The geometric mean MCYST concentration was 0.2 µg/L, 1.0 µg/L, and 0.8 µg/L by PPI, ELISA, LC-MS/MS, respectively.

MCYST levels in surface water globally, are summarized in Table 14. Mean MCYST in Alberta varied greatly with location, but are within a range of MCYST levels reported in the literature.

Because many cyanobacteria do not produce MCYST and free MCYST dissolved in the water varies depending on many factors such as depth of water, weather conditions, growth phase etc., the measured MCYST levels may not reflect the true extent of cyanobacteria blooming in the water (Akcaalan et al 2006, Davis et al 2009, Leblanc Renaud et al 2011, Sivonen 1990, Tonk et al 2005, Wiedner et al 2003).

Five MCYST variants were analyzed by LC-MS/MS in 2011 and 2012 (Table 15). MC-LR was the most predominant variant, accounted for 67 to 100 per cent of ∑MCYST in 2011 and 63 to 100 per cent of ∑MCYST in 2012. MC-LR was detected in 71 per cent and 40 per cent of samples in 2011 and 2012, respectively.

Nine MCYST variants were analyzed in 2013 (Table 15). MC-LR was the predominant variant in most lakes monitored, accounting for 52 to 100 per cent of ∑MCYST. MC-LA was predominant in Cochrane Lake and Muriel Lakes (58 to 100 per cent of ∑MCYST). MC-LY was predominant in Fork Lake (100 per cent of ∑MCYST). MC-LR, MC-LA and MC-LY were detected in 41 per cent, 22 per cent and 4 per cent of samples, respectively.

Water samples were mainly collected between June and September in 2010, 2011, 2012 and 2013. MCYST levels exceeding 20 µg/L (by PPI assay) were typically observed between July and September (Figure 12). In 2010, samples only collected in August exceeded MCYST levels greater than 20 µg/L. In 2011, samples collected in July, August and September greater than 20 µg/L, with the highest percentage of samples being collected in September. In 2012 and 2013, the percentage of samples greater than 20 µg/L gradually increased from July to September. These findings are in agreement with the suggestion that cyanobacterial blooms in Alberta's water bodies usually occur from mid-summer to early fall as water column stability required for surface bloom formation is greatest during this period (Zurawell 2010).

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Table 14 Reported MCYST Levels in Surface Water in Literature

Country	Water Body	Mean Conc. µg/L	Lab Method	Variant	Ref.
Bulgaria	Sofia region reservoirs	1.64	HPLC-PDA	LR 52%, RR 48%	(Pavlova et al 2006)
Germany	Bleiloch Reservoir	1.3	LC/MS		(Hummert et al 2001)
Lithuania	Curonian Lagoon	3.43	HPLC-PDA	RR 49% YR 7% LY 0.6%	(Paldavičienė et al 2009)
Netherlands	86 fresh surface waters	49	LC/MS	LR 55% RR 8% YR 3% LW 20% LF 6% LY 8%	(Faassen & Lurling 2013)
Spain	7 reservoirs, Madrid	15.7	HPLC-PDA		(Carrasco et al 2006)
	El Atazar Reservoir	3.0	LC/MS	LR 2% RR 97% YR 1%	(Barco et al 2004)
Argentina	San Roque Reservoir	2.17	HPLC-UV-MS/MS	LR 24% RR 73% YR 3%	(Ruiz et al 2013)
	Salto Grande Dam	48.6	HPLC-PDA		(Giannuzzi et al 2011)
	Los Padres Lake	7.6	HPLC-MS/MS	LR 2% RR 83% LA 14% YR 1%	(Amé et al 2010)
USA	Lake Mendota, Wisconsin	1.4	HPLC-MS/MS	LR 99.1% RR 0.2% YR 0.7%	(Beversdorf et al 2013)
Canada	9 Lakes, Alberta	0.4 - 26	LC/MS/MS	LR 100%	Zurawell 2010
	Driedmeat Lake	1.6	LC/MS/MS	LR 73% RR 27%	Zurawell 2010
	Thunder Lake	12.9	LC/MS/MS	LR 73% RR 27%	Zurawell 2010
	Twin Valley	76	LC/MS/MS	LR 91% RR 8% YR1%	Zurawell 2010
China	Qiantang River, West lake, Zhejiang	2.29	HPLC-MS/MS	LR 70% RR 30%	(Wang et al 2007)
	Lake Taihu, Jiangshu	0.78	LC-ESI-MS	YR 47%	(Zhang et al 2009)
	Lake Taihu, Jiangshu	0.8	HPLC-PDA	LR 63% RR 32% YR 5%	(Li et al 2012)

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(Continued)

Country	Water Body	Mean Conc. µg/L	Lab Method	Variant	Ref.
India	Durgakund , Varanasi	124.5	LC-MS	LR 40% RR 50% YR 10%	(Srivastava et al 2012)
Japan	Lake Sagami, Kanagawa	0.5	HPLC-UV	LR 14% RR 62% YR 24%	(Tsuji et al 1996)
Australia	Claremont Lake	14.5	HPLC		(Kemp & John 2006)
	Emu Lake	634	HPLC		(Kemp & John 2006)
	Booragoon Lake	26.1	HPLC		(Kemp & John 2006)
New Zealand	Lake Hakanoa	2.1	LC-MS/MS + ELISA	LR 25% RR 31% FR 15% WR 15%	(Crush et al 2008)
	Hokio Stream	41.6	LC-MS	LR 55% RR 45%	(Mountfort et al 2005)
	Lake Horowhenua	83	LC-MS	LR 54% RR 40% YR 6%	(Mountfort et al 2005)
Canada	Bécancour River Quebec	0.049	LC-MS/MS	LR 100%	(Robert et al 2004)
	Missisquoi Bay Quebec	0.48	LC-MS/MS	LR 67% MC-27% YR 6%	(Robert et al 2004)
	Yamaska River, Quebec	1.2	LC-MS/MS	LR 45% RR 49% YR 6%	(Robert et al 2004)
	Yamaska River, Quebec	0.37	LC-MS/MS	LR 100%	(Robert et al 2004)
	12 Lakes, Alberta	0.09 – 3.7	HPLC		(Kotak et al 2000)
	Lake Ontario, Ontario	32	HPLC+ELISA		(Murphy et al 2003)
USA	2 Lakes, Pensilvania	0.13	HPLC+ELISA		(Murphy et al 2003)
Czech Republic	Nove Mlyny Reservoir	1.06	ELISA		(Bláha et al 2010)
	70 reservoirs	0.88	ELISA		(Bláhová et al 2008)
Spain	Lake Albufera	1.70	ELISA		(Romo et al 2012)
	Alharabe River,	0.2	ELISA		(Aboal et al 2005)
	8 reservoirs Segura River	0.08	ELISA		(Aboal & Puig 2005)

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(Continued)

Country	Water Body	Mean Conc. µg/L	Lab Method	Variant	Ref.
Argentina	San Roque Reservoir	6.0	ELISA		(Conti et al 2005)
Brazil	Utinga Reservoir	0.65	ELISA		(Vieira et al 2005)
	Itaipu Lake	6.6	ELISA		(Hirooka et al 1999)
	Sao Miguel do Iguacu	10.0	ELISA		(Hirooka et al 1999)
USA	2 lakes, California	97.5	ELISA+LC/MS		(Backer et al 2010)
	187 lakes, Florida	0.4	ELISA		(Bigham et al 2009)
	177 reservoirs Missouri	0.23	ELISA		(Graham & Jones 2009)
	Falls Lake, North Carolina	0.16	ELISA		(Ehrlich & Gholizadeh 2008)
USA	Buffalo Springs Lake, Texas	0.92	ELISA+PPI		(Billam et al 2006)
	Lake Ransom Canyon,Texas	0.78	ELISA+PPI		(Billam et al 2006)
Canada	4 Lakes, Quebec	0.14 – 1.9	PPI		(Rolland et al 2005)
	21 Lakes, Quebec	0.002 – 1.9	PPI		(Giani et al 2005)
	8 Lakes, Alberta	0.01 – 5.0	PPI		(Zurawell 2002)
Serbia	Vojvodina reservoirs	59.5	PPI		(Svirčev & Simeunović 2013)
China	Donghu Lake, Wuhan	0.07	PPI		(Xu et al 2000)

Cyanobacterial blooms varied year to year and lake to lake. Figure 13 shows temporal trends in MCYST (detected by PPI) in Isle Lake and Eagle Lake over the 4 year period (2010 to 2013). Differences in nutrient availability, air and water temperatures, sunlight condition, and wind velocity can all contribute to spatial and the temporal differences in MCYST (Akcaalan et al 2006, Davis et al 2009, Leblanc Renaud et al 2011, Sivonen 1990, Tonk et al 2005, Wiedner et al 2003). Furthermore, the composition of cyanobacteria species causing a bloom the types and concentration of cyanotoxins produced (Dietrich et al 2008, Yepremian et al 2007).

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Table 15 Percentage of MCYST Variants

	2011 N=83			2012 N=562			2013 N=315		
	≥ 0.1 or 0.2*µg/L	% **	Range (µg/L)	≥ 0.1 or 0.2*µg/L	%	Range (µg/L)	≥ 0.1 or 0.2*µg/L	%	Range (µg/L)
MC-LR	59	71	nd – 59.5	224	40	nd – 171	130	41	nd – 143
MC-RR	32	39	nd – 3	47	8	nd – 6.7	6	2	nd – 0.9
MC-YR	2	2	nd – 0.3	4	1	nd – 1.8	1	0.3	nd – 0.7
MC-LW	3	4	nd – 1	1	0.2	nd – 0.2	0	0	nd
MC-LF	2	2	nd – 0.8	2	0.4	nd – 0.4	1	0.3	nd – 0.5
MC-LA	-	-	-	-	-	-	70	22	nd – 160
MC-LY	-	-	-	-	-	-	13	4	nd – 0.5
dMe-LR	-	-	-	-	-	-	5	2	nd – 1.5
MC-HtyR	-	-	-	-	-	-	3	1	nd – 3.4

*LOQ; nd: not detected.** % = percent of samples with detection of the variant shown

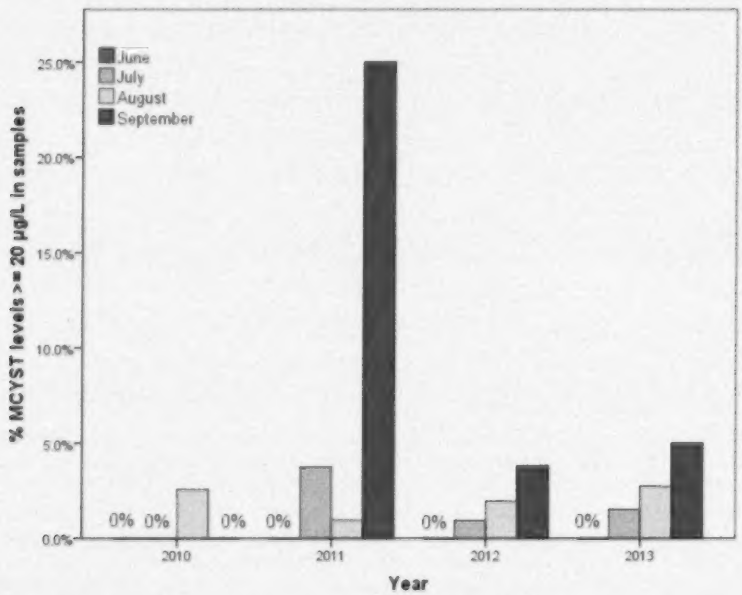

Figure 12 Temporal Trends of MCYST Levels ≥ 20 µg/L

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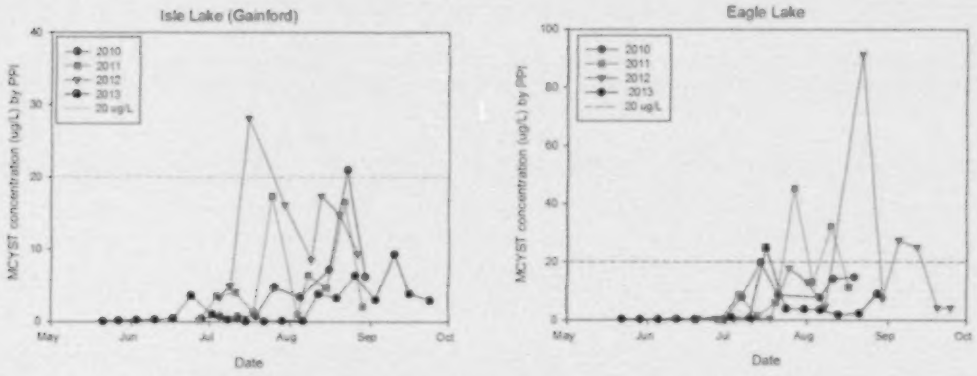

Figure 13 MCYST levels (PPI) in Two Lakes in 2010 – 2013

2.1.2 Cell Density

Total cyanobacterial cell count was conducted for 136 samples collected from 14 advisory lakes in 2012, and 265 samples collected from 45 lakes (including 20 advisory lakes) in 2013. Fifty-eight per cent and 27 per cent of samples had total cyanobacterial cell count exceeding 100,000 cells/mL in 2012 and 2013, respectively. Cell density exceeding the guideline values was frequently observed in June, July, August and September. Detailed results are summarized in Appendix C. Cyanobacteria genera and their cyanotoxin are summarized in Appendix G. The algae groups contained a number of species:

1. *Cyanophyceae* (Blue-greens): 105 species
2. *Chlorophyceae* (Greens): 155 species
3. *Euglenophyceae* (Euglenoids): 18 species
4. *Chrysophyceae* (Chrysopytes): 108 species
5. *Bacillariophyceae* (Diatoms): 184 species
6. *Cryptophytes* 24 species
7. *Pyrrophyceae* (*Dinoflagellates*): 37 species

The species known to produce liver toxin – MCYST (de Figueiredo et al 2004) are:

1. *Microcystis spp*
2. *Anabaena (An.) spp*
3. *Planktothrix (Oscillatoria (P. agardhii and P. rubescens)*
4. *Nostoc (N. rivulare)*
5. *Aphanizomenon (Aph. flos-aquae)*
6. *Hapalosiphon*
7. *Gloeotrichia (G. echinulata)*

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Not all cyanobacterial species produce MCYST. It is important to analyze the diversity of cyanobacteria contained in algal blooms in Alberta lakes. The numbers of species varied between lakes.

In 2012, a minimum of 2 species was observed in Calling and McLeod Lake. Thirty six species were observed in Pigeon Lake (Table 16). MCYST-producing species dominated in 14 advisory lakes, accounting for 46 to 100 per cent of the total cyanobacterial population (Figure 14).

In 2013, a minimum of 4 species was observed in Hasse Lake, Hastings Lake and Haunted Lake. Thirty two species were observed in Pigeon Lake (Table 17). Among 20 advisory lakes, MCYST-producing species dominated in 17 lakes, accounting for 58 to 100 per cent of the total cyanobacterial population (Figure 14). The detailed speciation for advisory lakes is summarized in Appendix C.

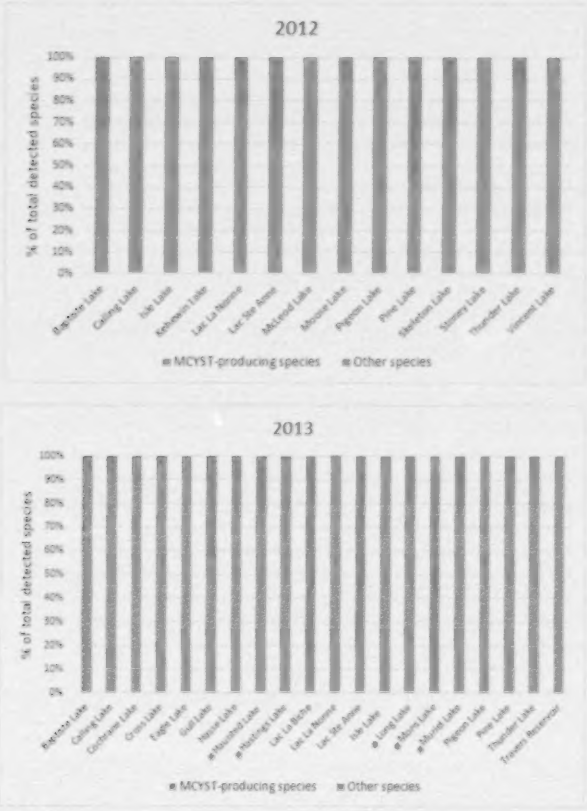

Figure 14 Proportion of MCYST vs Non-MCYST Producing Species

[Note: The sample with maximum total cyanobacterial population was selected to calculate the proportions in each lake. *: only one sample was collected.]

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Table 16 Total Species Number of Cyanobacteria in 14 Lakes in 2012

	Total	MCYST-Produced Species	Other Species
Baptiste Lake	8	2	6
Calling Lake	2	2	0
Isle Lake	19	7	12
Kehewin Lake	10	9	1
Lac La Nonne	16	8	8
Lac Ste Anne	4	2	2
McLeod Lake	2	2	0
Moose Lake	23	11	12
Pigeon Lake	36	15	21
Pine Lake	18	10	8
Skeleton Lake	5	3	2
Stoney Lake	4	3	1
Thunder Lake	13	6	7
Vincent Lake	7	5	2

Table 17 Total Species Number of Cyanobacteria in 20 Lakes in 2013

	Total	MCYST-Produced Species	Other Species
Baptiste Lake	19	5	14
Calling Lake	19	11	8
Cochrane Lake	8	8	0
Cross Lake	19	10	9
Eagle Lake	12	7	5
Gull Lake	17	7	10
Hasse Lake	4	3	1
Hastings Lake	4	3	1
Haunted Lake	4	3	1
Isle Lake	22	16	6
Lac La Biche	14	3	11
Lac La Nonne	11	5	6
Lac Ste Anne	8	6	2
Long Lake	7	5	2
Mons Lake	6	4	2
Muriel Lake	16	5	11
Pigeon Lake	32	14	18
Pine Lake	27	10	17
Thunder Lake	11	9	2
Travers Reservoir	10	9	1

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2.1.3 mcyE Levels

In 2012, a total of 527 water samples were tested by qPCR. *mcy*E was detected in 70 per cent of samples. High levels of *mcy*E (i.e. greater than 1.0×10^5 copies/mL) were found in 30 samples (8 per cent). *mcy*E gene was detected in 41 out of 45 lakes (Figure 15). Moderate to high *mcy*E levels (i.e. 500 – 3.4×10^6 copies/mL) were found in 31 lakes. Low *mcy*E levels (<500 copies/mL) were found in 10 lakes and no *mcy*E was detected in 4 lakes.

In 2013, a total of 599 water samples were tested by qPCR. *mcy*E was detected in 45 per cent of samples. High levels of *mcy*E (i.e. greater than 1.0×10^5 copies/mL) were found in 11 samples (4 per cent). *mcy*E gene was detected in 38 out 47 lakes (Figure 15). Moderate to high *mcy*E levels (i.e. 500 – 1.8×10^6 copies/mL) were found in 31 lakes. Low *mcy*E levels (<500 copies/mL) were found in 7 lakes and no *mcy*E was detected in 9 lakes.

The maximum levels of *mcy*E in all lakes are showed in Figure 16. Ten out of the 15 lakes and 4 out of the 20 lakes under the public health advisories showed high *mcy*E levels (> 1.0×10^5 copies/mL) in 2012 and 2013, respectively.

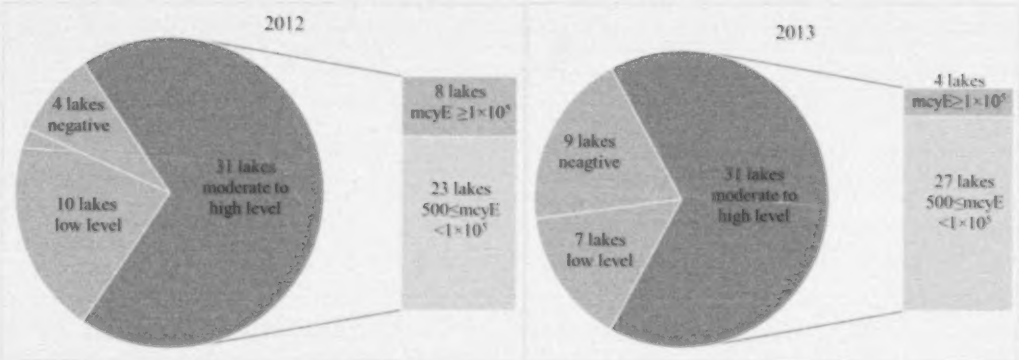

Figure 15 Distribution of *mcy*E Levels in Lakes in 2012 and 2013

As compared to the guideline value of 20 µg/L, *mcy*E copy number at e^{11} (59874) copies/mL could be used as a reference estimate of MCYST guideline exceedence in lakes (Table 18).

In summary, qPCR developed by Public Health Laboratory-Microbiology is a simple, rapid, sensitive, specific and quantitative assay, which can serve as a useful tool for monitoring cyanobacteria in water. The results demonstrated that the levels of *mcy*E were correlated to MCYST levels in the monitored lakes. This

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tool has potential for rapid screening, early detection and prediction of toxin-producing cyanobacteria in recreational water.

Table 18 Estimated Equivalence to MCYST 20 µ/L by Using qPCR

	N	R	R^2	p-value	Estimated equivalence to MCYST 20 µ/L by Using qPCR
qPCR vs. ELISA	336	0.59	0.35	<0.001	ln Copies = 7.9 + 0.9 ln 20= 10.5
qPCR vs PPI	708	0.66	0.42	<0.001	ln Copies = 8.5 + 1.0 ln 20=11.5
qPCR vs. LCMS	352	0.50	0.25	<0.001	ln Copies = 9.0 + 0.8 ln 20=11.4

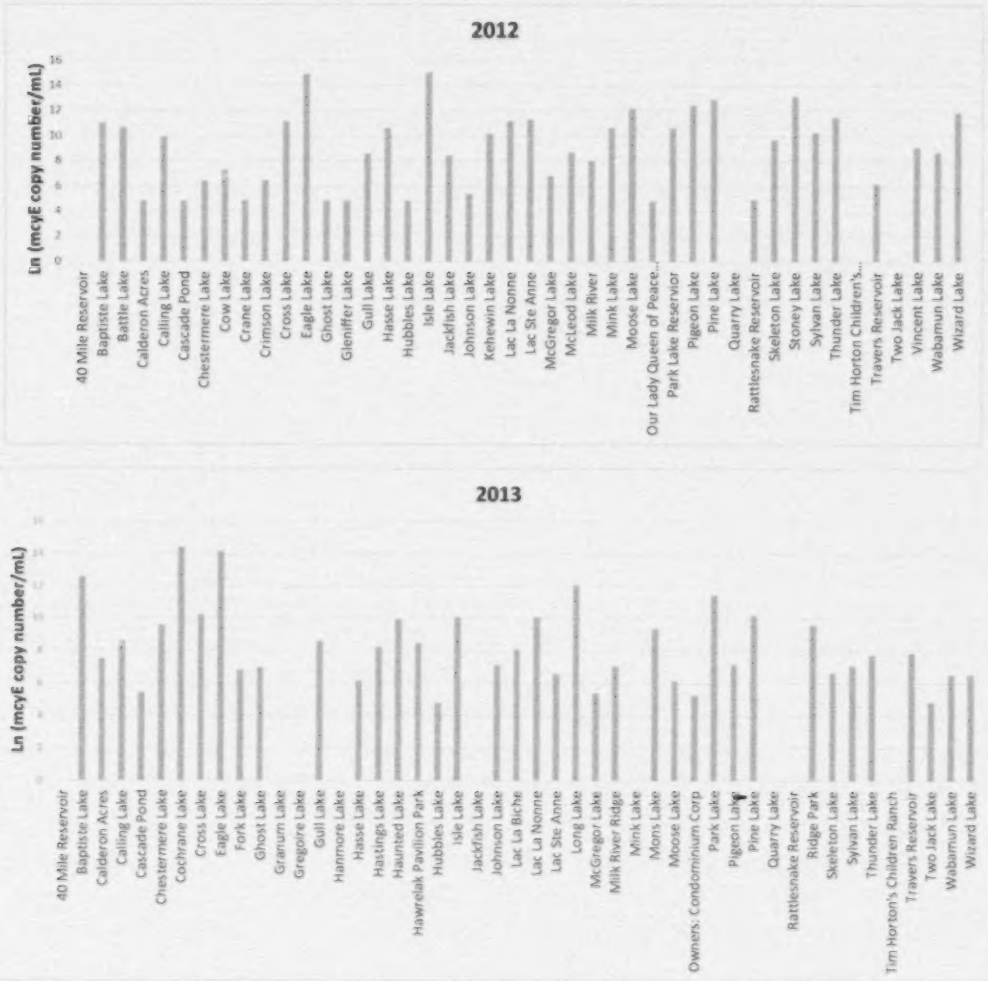

Figure 16 *mcy*E Levels in Lakes in 2012 and 2013

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2.1.4 Visual Inspection and Advisories

In 2012, public health advisories were issued for 17 recreational lakes according to visual inspection (Figure 17 and Table 19). Cell counting was conducted for 14 lakes. Cell density exceeded the guideline of 100,000 cells/mL in 14 lakes where the samples were collected for cell count analysis. MCYST levels periodically exceeded the recreational water guideline of 20 µg/L in 12 out of 17 lakes. Both MCYST and cell density exceeded guidelines in 9 lakes.

In 2013, public health advisories were issued for 35 recreational lakes (Figure 18). Off 35 lakes under advisories, the water samples were collected in 22 lakes (Table 20, Appendix A). PPI test was conducted for 22 lakes. Cell counting by using Automated FlowCam method was conducted for 20 lakes. Cell density exceeded 100,000 cells/mL in all lakes except for long lake. MCYST levels exceeded 20 µg/L in 7 out of 22 lakes. Both MCYST and cell density exceeded the guideline values in 7 lakes.

Table 19 Visual Inspection and Advisories in 2012

Lakes under the Advisory	PPI ≥ 20 µg/L	ELISA ≥ 20 µg/L	LC-MS/MS ≥ 20 µg/L	Cell count >100,000 cells/mL
Baptiste Lake		×		×
Calling Lake				×
Eagle Lake	×	×	×	NA
Gregoire Lake		×		NA
Isle Lake	×	×	×	×
Kehewin Lake	×	×	×	×
Lac La Nonne	×	×	×	×
Lac Ste Anne				×
McLeod Lake				×
Moonshine Lake	×	×		NA
Moose Lake	×	×		×
Pigeon Lake		×		×
Pine Lake				×
Skeleton Lake		×	×	×
Stoney Lake	×	×	×	×
Thunder Lake		×	×	×
Vincent Lake				×

×: water samples measured using laboratory methods exceeded the guideline values. Blank cells: water samples measured by the laboratory method did not exceed the guideline values. NA: data not available.

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Table 20 Visual Inspection and Advisories in 2013

Lakes under the Advisory	PPI ≥ 20 µg/L	ELISA ≥ 20 µg/L	LC-MS/MS ≥ 20 µg/L	Cell count >100,000 cells/mL
Baptiste Lake				×
Calling Lake				×
Cochrane Lake	×	×	×	×
Cross Lake				×
Eagle Lake	×	×	×	×
Fork Lake				NA
Gull Lake		NA		×
Hasse Lake				×
Hastings Lake		NA		×
Haunted Lake	×	×	×	×
Isle Lake	×	×	×	×
Lac La Biche		NA		×
Lac La Nonne	×	×	×	×
Lac Ste Anne				×
Long Lake		NA		
Mons Lake		NA	NA	×
Muriel Lake	×	×		×
Pigeon Lake				×
Pine Lake				×
Thunder Lake				×
Travers Reservoir	×	NA		×
Twin Valley Reservoir		NA		NA

×: water samples measured using laboratory methods exceeded the guideline values. Blank cells: water samples measured by the laboratory method did not exceed the guideline values. NA: data not available.

Advisories were issued in 29 lakes by using visual inspection. The accuracy of visual inspection was confirmed by using cell counts late for the lakes in which the samples were collected. Advisories were issued in 6 lakes based on cell counting greater than 100,000 cells/mL, while visual inspection did not capture significant color changes in water. These lakes were Baptiste Lake, Calling Lake, Cross Lake, Gull Lake, Lac La Biche and Pine Lake.

The results indicate that visual inspection serves a simple and fairly effective means for issuing the public health advisories.

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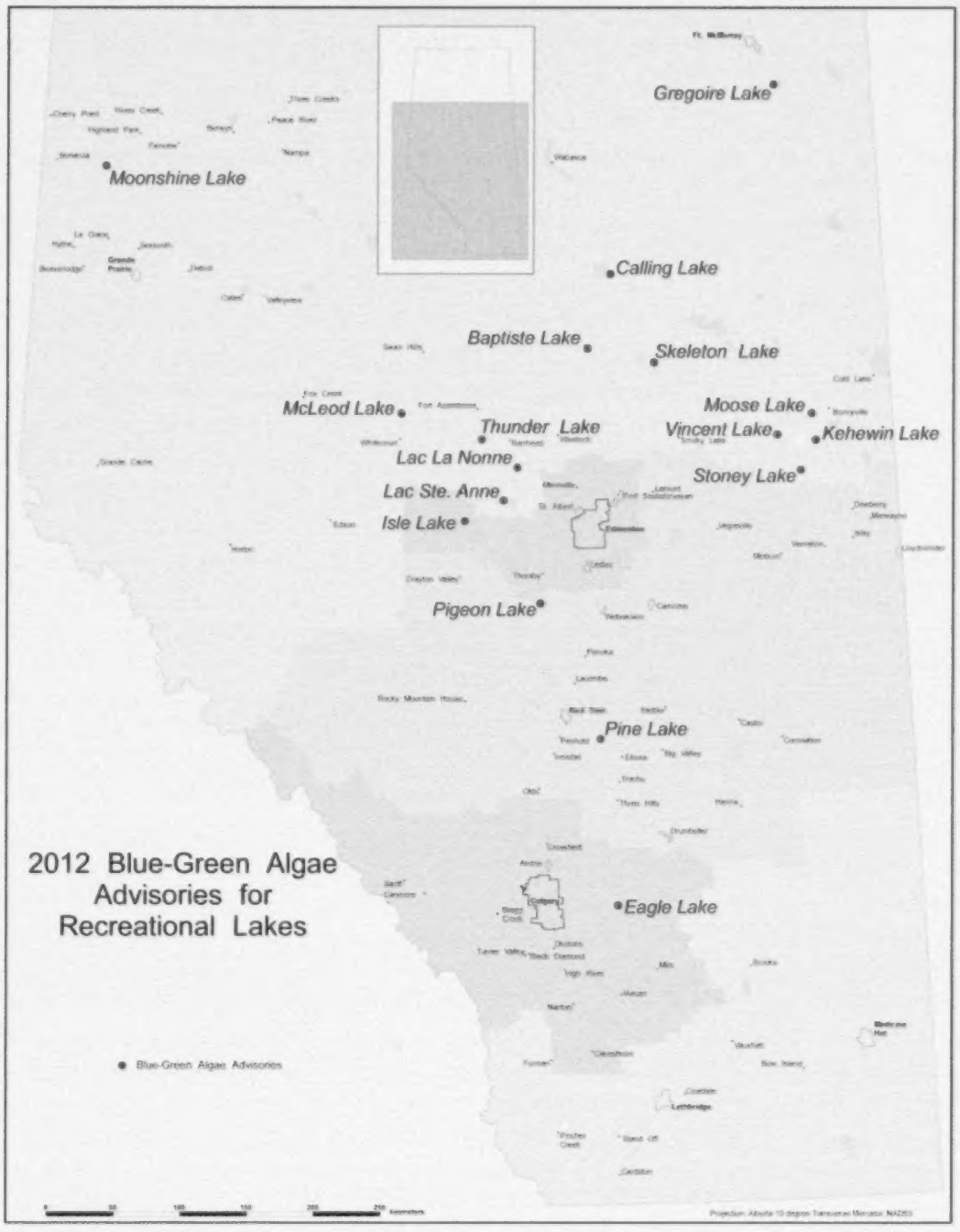

Figure 17 Lakes under Public Health Advisories in 2012

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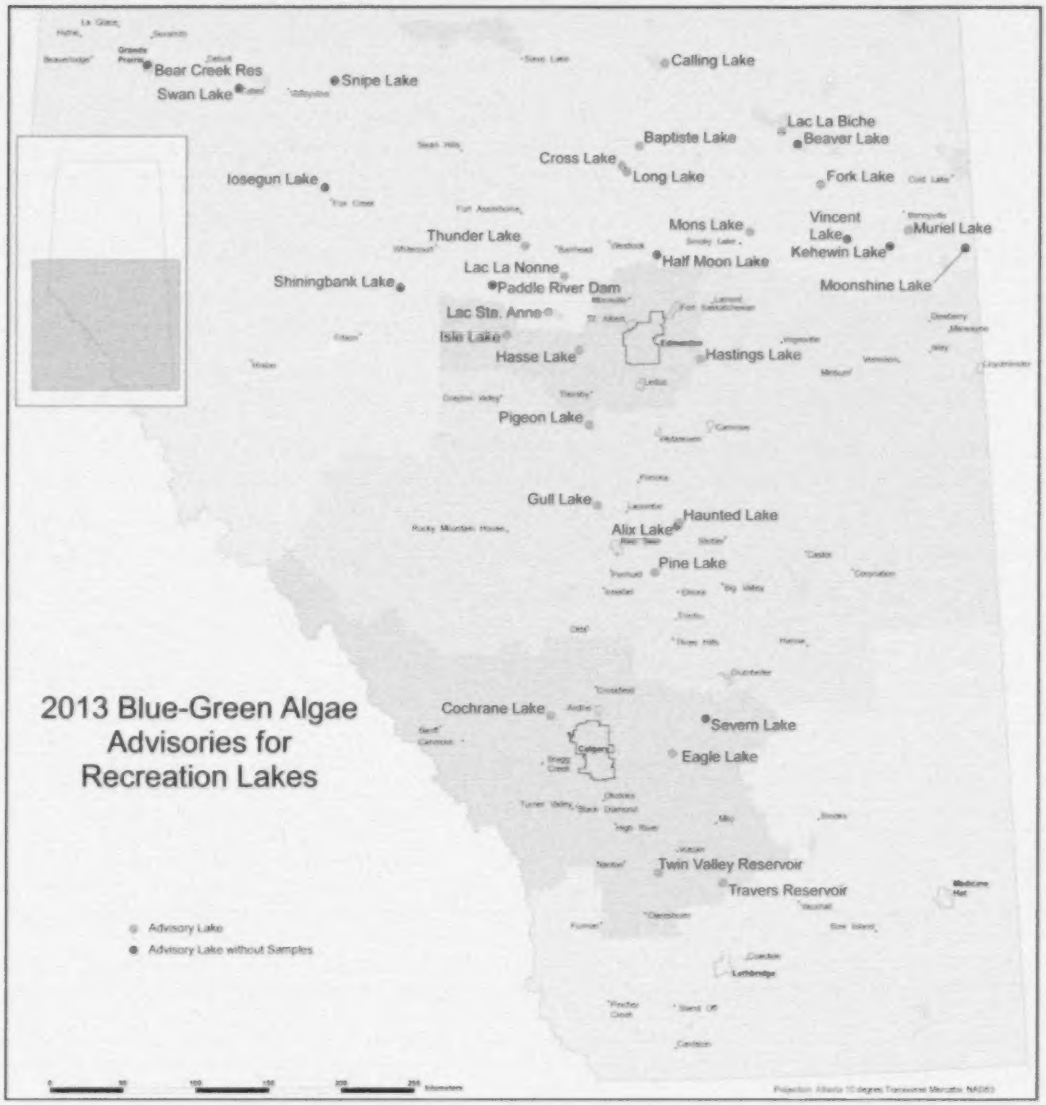

Figure 18 Lakes under Public Health Advisories in 2013

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2.2 Fish

In order to evaluate the level of MCYST in fish from Alberta lakes, fish were collected from a number of lakes that were under public health advisories as well as two control lakes without advisories. Of the 561 fish collected, 357 muscle samples were analyzed for MCYST. None of the muscle samples had detectable levels of MCYST present as quantified by LC-MS/MS. The findings revealed that MCYST does not accumulate appreciably in the tissue of fish present in Alberta lakes.

These results agree with results reported in other lakes in the United States (Johnson et al 2013, Prendergast & Foster 2010) that also used LC-MS/MS for MCYST quantitation. In comparison with the 14 ng/g provisional guideline value in use in Alberta, the results suggest that there is no significant risk associated with the consumption of fish muscle (fillet) from lakes in Alberta with advisories.

LC-MS/MS measures concentrations of specific congeners of MCYST. LC-MS/MS is limited insofar that only those congeners, which analytical standards are available can be quantified (Mekebri et al 2009). It is therefore possible that congeners for which standards were not available and may have been present in the extracts prepared, but not measured. Furthermore, LC-MS/MS is only one technique that has been used to quantify MCYST in tissue extracts. In the body of literature investigating MCYST uptake into fish tissues. ELISA is more frequently used than LC-MS/MS. ELISA typically yields higher MCYST values than LC-MS/MS (Johnson et al 2013), and in theory should be able to quantify all congeners of MCYST at the same time. However, ELISA is unable to differentiate between individual congeners (Harada et al 1999). Because of it lack of commercial availability, PPI has been used less frequently in the analysis of MCYST in tissue, but is sometimes used in conjunction with other methods (Berry et al 2011).

Despite the difficulty associated with the analysis of MCYST in fish tissue, the degree of risk associated with consumption of fish from lakes with blue-green algal blooms in Alberta appears low. This is in line with expert commentary on the subject matter: "the consumption of fish muscle tissue cannot be considered a major hazard to human health but there are fish species and/or fish organs which may accumulate significant amounts of toxins" (Meriluoto & Spoof 2008). There have been no reported cases of illness in Alberta from people consuming MCYST contaminated fish from these lakes.

It is important to note that results pertaining to liver tissue analysis are forthcoming and therefore comment regarding toxicity and possible risk to consuming whole fish is yet to come. It is expected that a higher proportion of MCYST would accumulate in liver tissue as this is the target organ of MCYST toxicity, but this increased hazard is mollified by the fact that the liver constitutes

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a small portion of the actual fish, and many people discard fish viscera and trimmings. When such accumulations become very high, liver structure begins to break down and hemorrhaging occurs that will leave the liver looking abnormal for such extreme cases, so a general warning for anyone inclined to eat fish livers is to avoid them if they look unusually bloody.

Furthermore, our analytical technique has not yet been validated against a complementary technique such as ELISA or PPI – concordance between results from different techniques would add an additional degree of confidence to our results.

Reconsideration of the current messaging for Albertans regarding fish consumption from lakes with blue-green algae advisories may be warranted if levels higher than our current guideline value (14 ng/g) are observed, or if new evidence suggests that a human health risk exists at lower concentrations.

3. Conclusions

All lakes under advisories issued based on visual confirmation of bloom showed either total cyanobacterial density or MCYST levels exceeding the Health Canada recreational water guidelines during period of monitoring. This finding indicates that the visual inspection method is an effective practice for risk management in terms of speed and communication with public, but it is known to be a subjective and imperfect screening method because factors including wind direction, below surface and diffuse blooms, and inspector/sampler error could contribute to situations where blooms (and possibly toxicity) exist but are not detected and advisories are not issued.

The cell density of cyanobacteria exceeded the guideline in all lakes under public health advisories except for one lake, when the samples were collected and submitted for cell counting. The types of total cyanobacterial species in these lakes varied from one lake to another. MCYST-producing species were dominating in most lakes. The peaks of total cyanobacteria blooming were typically observed in late August and September in most lakes. Most occurrences of cyanobacteria blooms exceeding guidelines were found in northern, central and Edmonton zones. Cell counting is a useful method for determining extent and types of species of blue-green algae blooms. It provides valuable information to support the issuance of public health advisories.

MCYST exceeded guidelines in some lakes under public health advisories, but was not concomitant with the increased cell density in most cases. PPI and ELISA methods are considered useful for screening purposes and LC-MS/MS for confirmatory purposes, but they do not provide timely information for issuing public health advisories in this monitoring program. The MCYST results could

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assist with evaluating the effectiveness of risk management after issuing public health advisories.

qPCR is a newly developed method. The level of *mcy*E has the potential to be used as an advance prediction for the level of cyanobacterial blooms in some lakes.

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Part III

Public Health Management

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From 2010-2013, AHS used visual inspection results to issue public health advisories for cyanobacterial blooming in the recreational water. The findings in 2012 and 2013 indicated that visual inspection was an effective way for risk management. In 2012 and 2013, cross-ministry initiative was chaired by Alberta Health working on the response to cyanobacterial blooms at Alberta recreational beaches. Membership includes:

1. Alberta Health Services
2. Alberta Environment and Sustainable Resource Development
3. Alberta Tourism, Parks and Recreation
4. Health Canada (FNIHB)
5. Alberta public health laboratories
6. Academic scientists

The purposes of the ACMPPH in the field of management are to:

1. better understand the public health risk posed by cyanobacterial blooms,
2. develop best practices for monitoring and issuing public health advisories for cyanobacterial blooms in Alberta,
3. improve cross-ministry coordination and alignment of work related to lake monitoring,
4. support research on early detection of blooms
5. generate recommendations for updates/improvements to policy based on field season data, and
6. enhance knowledge transfer, and risk communication.

The scope of the ACMPPH working group is to evaluate:

1. the scientific and operational implications for public health advisories, and
2. any scientific findings that may emerge from routine ACMPPH sampling that have public health implications (e.g., for municipal drinking water) are referred to the appropriate organization or program area.

The management process is illustrated in Figure 19. In 2012 and 2013, the working group (1) provided technical support and leadership to AHS in development of a practical, scientific, health-based public health advisory process for cyanobacterial blooms in recreational waters, (2) developed a sampling and analysis protocol based on concentration measurements of microcystin (one of the common toxins) and total cyanobacterial cells in the water, (3) compared to health-based guidelines for recreational water to evaluate the issuance of public health advisories through the practice of visual inspection.

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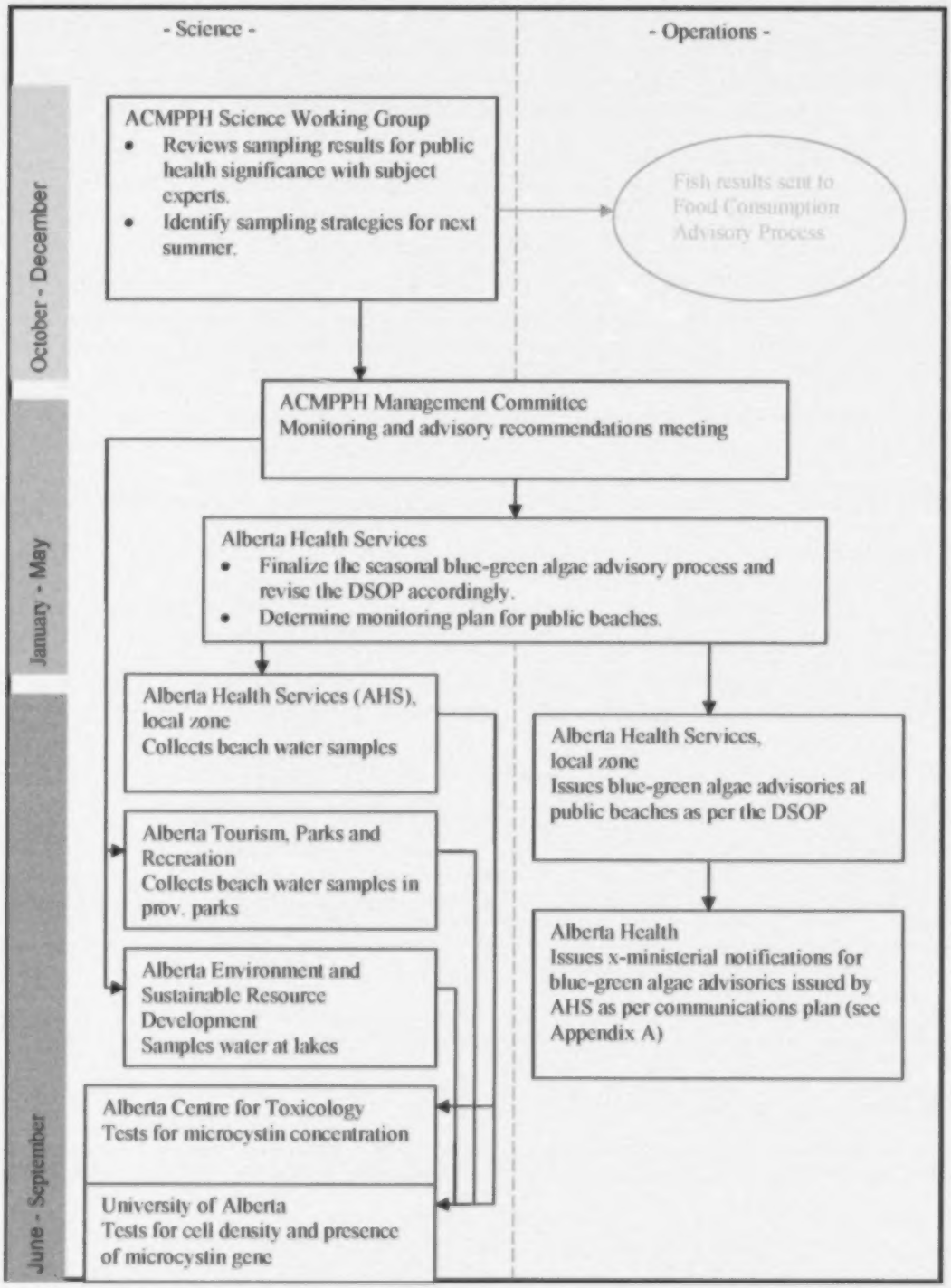
- Science -
- Operations -
October - December
January - May
June - September
ACMPPH Science Working Group
• Reviews sampling results for public health significance with subject experts.
• Identify sampling strategies for next summer.
Fish results sent to Food Consumption Advisory Process
ACMPPH Management Committee
Monitoring and advisory recommendations meeting
Alberta Health Services
• Finalize the seasonal blue-green algae advisory process and revise the DSOP accordingly.
• Determine monitoring plan for public beaches.
Alberta Health Services (AHS), local zone
Collects beach water samples
Alberta Health Services, local zone
Issues blue-green algae advisories at public beaches as per the DSOP
Alberta Tourism, Parks and Recreation
Collects beach water samples in prov. parks
Alberta Health
Issues x-ministerial notifications for blue-green algae advisories issued by AHS as per communications plan (see Appendix A)
Alberta Environment and Sustainable Resource Development
Samples water at lakes
Alberta Centre for Toxicology
Tests for microcystin concentration
University of Alberta
Tests for cell density and presence of microcystin gene

Figure 19 Management Process

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The Science Advisory Committee (SAC) developed recommendations that inform cyanobacteria monitoring and the public health advisory process. The recommendations developed by the SAC based on data collected have been provided to AHS to inform its sampling locations and protocol in next year.

The recommendations are to

1. continue routine monitoring for cyanobacteria, including confirmatory testing,
2. continue the shared approach for sample collection with Alberta Health Services, Alberta Centre for Toxicology, Alberta Environment and Sustainable Resource and Development, and Alberta Tourism, Parks and Recreation,
3. select priority lakes for systematic weekly monitoring where possible (May – Oct),
4. monitor new lakes with blue-green algae blooms, and
5. continue science-based monitoring program to improve advisory practice and communicate specific risks to an interested and informed public.

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Appendix A

Sampling Locations

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Sampling Locations in 2010, 2011, 2012, 2013

2010 N = 39	2011 N = 33	2012 N = 47	2013 N=49
Auburn Bay	Baptiste Lake	40 Mile Reservoir	40 Mile Reservoir
Baptiste Lake	Black Nugget Lake	Baptiste Lake	Baptiste Lake
Beaver Lake	Buffalo Lake	Battle Lake	Calderon Acres
Bonavista Lake	Calderon Acres	Calderon Acres	Calling Lake
Buffalo Lake	Calling Lake	Calling Lake	Cascade Pond
Chaparral Lake	Chestermere Lake	Cascade Pond	Chestermere Lake
Chestermere Lake	Cross Lake	Chestermere Lake	Cochrane Lake
Dried Meat Lake	Eagle Lake	Cow Lake	Cross Lake
Eagle Lake	Elkwater Lake	Crane Lake	Eagle Lake
Gull Lake	Ghost Lake	Crimson Lake	Fork Lake
Half Moon Lake	Golden Eagle Pond 2	Cross Lake	Ghost Lake
Hasse Lake	Gregoire Lake	Eagle Lake	Granum Lake
Hastings Lake	Gull Lake	Ghost Lake	Gregoire Lake
Isle Lake	Hasse Lake	Gleniffer Lake	Gull Lake
Islet Lake	Isle Lake	Gregoire Lake	Hanmore Lake
Jackfish Lake	Jackfish Lake	Gull Lake	Hasse Lake
Lac La Biche	Lac La Nonne	Hasse Lake	Hastings Lake
Lac La Nonne	Lac Sante	Hubbles Lake	Haunted Lake
Lac Ste Anne	Lac Ste Anne	Isle Lake	Hawrelak Pavilion Park
Little Beaver Lake	Little Bow Lake Reservoir	Jackfish Lake	Hubbles Lake
Little Bow Lake Reservoir	Maqua Lake	Johnson Lake	Isle Lake
McGregor Lake	McGregor Lake	Kehewin Lake	Jackfish Lake
McKenzie Lake	Our Lady Queen of Peace Ranch	Lac La Biche	Johnson Lake
Midnapore Lake	Park Lake Reservoir	Lac La Nonne	Lac La Biche
Miquelon Lake	Pigeon Lake	Lac Ste Anne	Lac La Nonne
North Buck Lake	Pine Lake	McGregor Lake	Lac Ste Anne
Our Lady Queen of Peace Ranch	Rattlesnake Reservoir	McLeod Lake	Long Lake
Pigeon Lake	Ridge Reservoir	Milk River	McGregor Lake
Pine Lake	Sylvan Lake	Mink Lake	Milk River Ridge
Sikome Lake	Thunder Lake	Moonshine Lake	Mink Lake
Skeleton Lake	Travers Reservoir	Moose Lake	Mons Lake
Spring Lake	Wabamun Lake	Our Lady Queen of Peace Ranch	Moose Lake
Steele Lake	Wizard Lake	Park Lake Reservior	Muriel Lake
Sundance Lake		Pigeon Lake	Owners: Condominium Corp
Sylvan Lake		Pine Lake	Park Lake

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Thunder Lake	Quarry Lake	Pigeon Lake
Travers Reservoir	Rattlesnake Reservoir	Pine Lake
Wabamun Lake	Skeleton Lake	Quarry Lake
Wizard Lake	Stoney Lake	Rattlesnake Reservoir
	Sylvan Lake	Ridge Park
	Thunder Lake	Skeleton Lake
	Tim Horton Children's Ranch	Sylvan Lake
	Travers Reservoir	Thunder Lake
	Two Jack Lake	Tim Horton Children's Ranch
	Vincent Lake	Travers Reservoir
	Wabamun Lake	Twin Valley Reservoir
	Wizard Lake	Two Jack Lake
		Wabamun Lake
		Wizard Lake

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Advisory Lakes without samples in 2013

Advisory Lakes without samples N=13	Issue date 2013
Alix Lake	9-Aug
Bear Creek and Reservoir	17-Jul
Beaver Lake	6-Sep
Half Moon Lake	29-Aug
Iosegun Lake	19-Jul
Kehewin Lake	8-Aug
Moonshine Lake	2-Aug
Paddle River Dam	28-Aug
Severn Lake	2-Aug
Shiningbank Lake	22-Jul
Snipe Lake	9-Jul
Swan Lake	15-Aug
Vincent Lake	9-Sep

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Appendix B

Acceptable Criteria for PPI, ELISA and LC-MS/MS Assays

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Acceptable QA/QC Criteria for PPI Assays

Control	Range (µg/L)	Use	Expected outcome/Action
Positive control (calibrators) MC-LR	0.05, 0.10, 0.21, 0.35, 0.48, 0.62	Each concentration run duplicates in every plate	CV% of the calibrator duplicates must be within ± 5% of the average values. Otherwise the results were rejected.
Negative sample control		Duplicate for every sample	CV% of the negative sample control duplicates must be within ± 5% of the average values. Otherwise the results were rejected.
100% Enzyme activity control		Duplicates in every plate	CV% of 100% enzyme activity controls duplicates must be within ± 5% of the average values. Otherwise the results were rejected.
Quality control sample (Previous year water sample with known MC-LR concentration)	0.24 – 0.34	Every plate	Value should fall within the expected range. Otherwise the results were rejected.
Check sample, MC-LR (Abraxis)	A < 0.15 B = 2 ± 0.5 C = 20 ± 5	Choose one sample to test in every plate (rotate from A to C)	Value should fall within the expected range, otherwise try problem solving and/or the results were rejected.

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Acceptable QA/QC Criteria for ELISA Assays

1. Control	Range (µg/L)	Use	Expected outcome/ Action
Positive control (calibrators) MC-LR	0, 0.15, 0.4, 1, 2 and 5	Each concentration run duplicates in every plate	Average %CV of each entire plate was less than 10%, otherwise the results are rejected.
Positive control, Known MC-LR concentration (include in the kit, Abraxis)	0.750 ± 0.185	Duplicate in every plate	Value should fall in the expected range, otherwise the results are rejected.
Negative control (ddH_2O)	0	Duplicate in every plate	Not detected or very low concentration detected at the level <0.1 µg/L, otherwise the results are rejected.
Check sample, MC-LR (Abraxis)	A < 0.15 B = 2 ± 0.5 C = 20 ± 5	Either sample A, B, or C in every plate	Value should fall in the expected range, otherwise try problem solving and/or reject the results

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Acceptable QA/QC Criteria for LC-MS/MS Assays

Control	Range (µg/L)	Use	Expected outcome/Action
Positive control (calibrators) MC-LR	0.1, 0.2, 0.5, 1, 5, 10, 20	Run in every batch	A chromatographic peak is acceptable if peak shape is symmetrical. The control levels must be ± 20% of the target values, otherwise the whole batch had to be repeated. The MRM ratios for the target compound and internal standard in the samples and control must be within ± 20% of the ion ratios in the Calibrator. Retention time (RT) for the target compound in the calibrator, samples and control must be within ± 3% of established values. Retention time (RT) for the internal standard in the calibrator, samples and control must be within ± 3% of established values. Relative retention time (RRT) of the target compound in the samples and control must be within ± 3% of those in the Calibrator. If the autosampler should fail in the middle of the run, samples that were not bracketed by controls shall be re-injected along with the Calibrators and Controls.
Internal QC: Spike samples For MC-LR, -RR, -YR, -LW, -LF	1.0 (each MCYST analogue)	Run after calibrators, in the middle of the sequence and at the end of sequence	
External controls: Check sample, MC-LR (Abraxis)	B = 2 ± 0.5	Run in every batch	

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Appendix C

Summary of Cell Counting Information

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Total Cyanobacterial Species in 14 Lakes in 2012

	MCYST-produced Species	Other Species
Baptiste Lake	*Microcystis aeruginosa Kutzing* *Oscillatoria sp.*	*Aphanizomenon flos-aquae Ralfs and Born.* *Chroococcus minutus (Kutzing) Nageli* *Cyanobium sp.* *Gomphosphaeria sp.* *Pseudoanabaena sp.*
Calling Lake	*Microcystis aeruginosa Kutzing* *Anabaena sp.*	
Kehewin Lake	*Anabaena circinalis Rabenhorst* *Anabaena cylindrica Lemmermann* *Anabaena planctonica Brunnthaler* *Anabaena sp.* *Anabaena spiroides Klebs* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis wesenbergii* *Oscillatoria sp.* *Planktolyngbya limnetica*	*Synechococcus sp.*
Lac La Nonne	*Anabaena circinalis Rabenhorst* *Anabaena flos-aquae intermedia* *Anabaena planctonica Brunnthaler* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis wesenbergii* *Oscillatoria sp.*	*Aphanocapsa sp.* *Chroococcus limneticus Lemmermann* *Chroococcus minutus (Kutzing) Nageli* *Coelospaerium Naegelianum Unger* *Cyanodictyon sp.* *Merismopedia sp.* *Pseudoanabaena sp.* *Synechococcus sp.*
Lac Ste Anne	*Microcystis aeruginosa (Kutzing) Kutzing* *Oscillatoria sp.*	*Cyanodictyon sp.* *Gomphosphaeria aponina Kutzing*
Isle Lake	*Anabaena flos-aquae intermedia* *Anabaena sp.* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa Kutzing* *Microcystis wesenbergii* *Oscillatoria sp*	*Aphaanizomenon sp* *Aphanizomenon insselshenkei* *Chroococcus sp.* *Coelospaerium kuetzingianum Nageli* *Coelospaerium Naegelianum Unger* *Cyanobium sp.* *Cyanodictyon sp.* *Pseudoanabaena raphidiodes* *Pseudoanabaena sp.* *Rhabdoderma sp.* *Spirulina sp. [=]* *Synechococcus sp.*
McLeod Lake	*Microcystis flos-aquae (Wittr.) Kirchn* *Oscillatoria sp.*	
Moose Lake	*Anabaena circinalis Rabenhorst* *Anabaena cylindrica Lemmermann* *Anabaena flos-aquae intermedia* *Anabaena sp. a* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and*	*Akinetes* *Aphanothece clathrata W. and G.S. West* *Cyanodictyon sp.* *Cylindrospermopsis sp.* *Fil. blue greens*

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	Born. *Gleoetrichia sp.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis viridis (Brunnthaler) Lemmermann* *Oscillatoria sp.* *Oscillatoria tenuis*	*Gomphosphaeria aponina Kutzing* *Merismopedia tenuissima Lemmermann* *Pseudoanabaena sp.* *Spirulina laxa G.M. Smith* *Spirulina laxissima* *Spirulina sp. [=]* *Synechococcus sp.*
Pigeon Lake	*Anabaena circinalis Rabenhorst* *Anabaena cylindrica Lemmermann* *Anabaena flos-aquae intermedia* *Anabaena planctonica Brunnthaler* *Anabaena skuja* *Anabaena sp.* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Gleoetrichia sp.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Oscillatoria sp.* *Planktolyngbya limnetica* *Planktolyngbya taillingii* *Planktothrix agardhii (Gom.) Anagnostidis and Komarek*	*Akinetes* *Chroococcus sp.* *Coelsphaerium sp.* *Cyanobium sp.* *Cyanodictyon sp.* *Eucapsis sp* *Fil. blue greens* *Gloeocapsa punctata* *Gomphosphaeria aponina Kutzing* *Heterocysts* *Merismopedia glauca (Ehrenberg) Kutzing* *Merismopedia minima (Ehrenberg) Kutzing* *Phormidium tenue (Ag. and Gom.) Anagnostidis and Komarek* *Plectonema sp.* *Pseudoanabaena raphidiodes* *Pseudoanabaena sp.* *Rhabdogloea lineare Schmidle and Lauterborn* *Rivularia sp.* *Small blue greens* *Synechococcus sp.*
Pine Lake	*Anabaena flos-aquae intermedia* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Gleoetrichia sp.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Microcystis viridis (Brunnthaler) Lemmermann* *Oscillatoria sp.* *Oscillatoria tenuis* *Planktolyngbya limnetica*	*Gomphosphaeria aponina Kutzing* *Gomphosphaeria lacustris var. compacta (Lemmermann)* *Gomphosphaeria sp.* *Lyngbya Birgei* *Lyngbya sp.* *Pseudoanabaena raphidiodes* *Pseudoanabaena sp.* *Synechococcus sp.*
Skeleton Lake	*Anabaena sp.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis wesenbergii*	*Coelsphaerium sp.* *Gomphosphaeria natus Komarek and Hindak*
Stoney Lake	*Microcystis aeruginosa Kutzing* *Oscillatoria sp.* *Planktolyngbya limnetica*	*Chroococcus sp.*
Thunder Lake	*Anabaena flos-aquae intermedia* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa (Kutzing)*	*Chroococcus sp.* *Cyanobium sp.* *Cylindrospermum sp.* *Merismopedia minima (Ehrenberg)*

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	Kutzing *Microcystis wesenbergii* *Oscillatoria sp.* *Planktolyngbya limnetica*	*Kutzing* *Merismopedia sp.* *Small blue greens* *Spirulina sp. [=]*
Vincent Lake	*Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis viridis (Brunnthaler) Lemmermann* *Microcystis wesenbergii* *Oscillatoria sp.* *Planktolyngbya limnetica*	*Chroococcus sp.* *Merismopedia minima (Ehrenberg) Kutzing*

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Total Cyanobacterial Species in 20 Lakes in 2013

	MCYST-produced Species	Other Species
Baptiste Lake	*Aphanizomenon flos-aquae Ralfs and Born.* *Aphanizomenon klebhnii* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Oscillatoria sp.*	*Aphanothece sp.* *Chroococcus sp.* *Cyanobium sp.* *Cyanodictyon sp.* *Fil. blue greens* *Gomphosphaeria aponina Kutzing* *Lyngbya sp.* *Phormidium sp* *Phormidium tenue (Ag. and Gom.) Anagnostidis and Komarek* *Pseudoanabaena sp.* *Small blue greens* *Woronichinia naegelianum (Unger) Elenk.* *Woronichinia robusta*
Calling Lake	*Anabaena circinalis Rabenhorst* *Anabaena crassa* *Anabaena cylindrica Lemmermann* *Anabaena flos-aquae (Lyngbye) Brebisson* *anabaena sp. a* *Aphanizomenon flos-aquae Ralfs and Born.* *Aphanizomenon gracile (Lemmermann) Lemmermann* *Aphanizomenon klebhnii* *Aphanizomenon sp.* *Gloeotrichia echinulata* *Oscillatoria sp.*	*Aphanocapsa sp.* *Chroococcus sp.* *Coelsphaerium sp.* *Fil. blue greens* *Phormidium sp* *Pseudoanabaena sp.* *Rhabdoderma sp.* *Synechococcus sp.*
Cochrane Lake	*Anabaena circinalis Rabenhorst* *Anabaena flos-aquae (Lyngbye) Brebisson* *anabaena sp. a* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Microcystis sp.* *Microcystis viridis (Brunnthaler) Lemmermann*	
Cross Lake	*Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena perturbata* *Anabaena planctonica Brunnthaler* *anabaena sp. a* *Anabaena sp. b* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Gleoetrichia sp.* *Oscillatoria sp.*	*Aphanothece sp.* *Chroococcus sp.* *Cyanobium sp.* *Cyanodictyon sp.* *Fil. blue greens* *Merismopedia punctata Meyen* *Phormidium sp* *Pseudoanabaena sp.* *Small blue greens*

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	Oscillatoria tenuis	
Eagle Lake	*Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena flos-aquae v. intermedia* *anabaena sp. a* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis sp.* *Oscillatoria sp.*	*Gomphosphaeria sp.* *Merismopedia minima (Ehrenberg) Kutzing* *Merismopedia tenuissima Lemmermann* *Pelogoea sp.* *Pseudoanabaena sp.*
Gull Lake	*Anabaena sp. (unknown)* *Anabaena spiroides Klebs* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Microcystis sp.* *Microcystis wesenbergii* *Oscillatoria tenuis*	*Aphanocapsa sp.* *Aphanothece sp.* *Chroococcus sp.* *Cyanobium sp.* *Gomphosphaeria aponina Kutzing* *Gomphosphaeria lacustris* *Gomphosphaeria sp.* *Lyngbya sp.[=Birgei]* *Woronichinia naegelianum (Unger) Elenk.* *Woronichinia robusta*
Hasse Lake	*Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.*	*Woronichinia naegelianum (Unger) Elenk.*
Hastings Lake	*Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis wesenbergii* *Oscillatoria sp.*	*Lyngbya confervicola*
Haunted Lake	*Aphanizomenon flos-aquae Ralfs and Born.* *Aphanizomenon gracile (Lemmermann) Lemmermann* *Microcystis aeruginosa (Kutzing) Kutzing*	*Phormidium mucicola*
Isle Lake	*Anabaena crassa* *Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena flos-aquae v. intermedia* *Anabaena perturbata* *Anabaena planctonica Brunnthaler* *Anabaena solitaria v. planctonica Brunnthaler* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Microcystis viridis (Brunnthaler) Lemmermann* *Microcystis wesenbergii* *Microcystis woronichinia*	*Cyanodictyon sp.* *Merismopedia minima (Ehrenberg) Kutzing* *Phormidium mucicola* *Phormidium sp* *Pseudoanabaena sp.* *Small blue greens*

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	Oscillatoria sp. *Oscillatoria sp. A* *Oscillatoria sp.B*	
Lac La Biche	*Anabaena flos-aquae* *Anabaena flos-aquae (Lyngbye) Brebisson* *Oscillatoria sp [thin-2-3um or Lyngbya-Phormidium]*	*Aphanocapsa delicatissima W. and G.S. West* *Aphanocapsa sp.* *Aphanothece sp.* *Chroococcus minutus (Kutzing) Nageli* *Chroococcus sp.* *Coelospaerium kuetzingianum Nageli* *Cyanobium sp.* *Cyanodictyon sp.* *Lyngbya sp.* *Merismopedia sp.* *Small blue greens*
Lac La Nonne	*Anabaena flos-aquae* *Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena spiroides Klebs* *Gloeotrichia echinulata* *Microcystis aeruginosa (Kutzing) Kutzing*	*Aphanocapsa sp.* *Aphanothece sp.* *Chroococcus limneticus Lemmermann* *Chroococcus minutus (Kutzing) Nageli* *Merismopedia sp.* *Small blue greens*
Lac Ste Anne	*Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena perturbata* *Anabaena planctonica Brunnthaler* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Gleoetrichia sp. (=pisum)*	*Aphanothece sp.* *Cyanodictyon sp.*
Long Lake	*Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena perturbata* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis viridis (Brunnthaler) Lemmermann* *Microcystis woronichinia*	*Phormidium mucicola* *Woronichinia robusta*
Mons Lake	*Anabaena perturbata* *Microcystis viridis (Brunnthaler) Lemmermann* *Microcystis wesenbergii* *Microcystis woronichinia*	*Cyanobium sp.* *Phormidium mucicola*
Muriel Lake	*Anabaena flos-aquae v. intermedia* *Anabaena spiroides Klebs* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Microcystis viridis (Brunnthaler) Lemmermann*	*Aphanocapsa sp.* *Chroococcus sp.* *Cyanobium sp.* *Cyanodictyon sp.* *Fil. blue greens* *Gloeocapsa sp.* *Gomphosphaeria aponina Kutzing* *Merismopedia glauca (Ehrenberg) Kutzing* *Merismopedia sp.* *Rhabdogloea lineare Schmidle and*

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		Lauterborn *Small blue greens*
Pigeon Lake	*Anabaena cylindrica Lemmermann* *Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena lemmermannii Usacev* *Anabaena perturbata* *Anabaena skuja* *Anabaena solitaria Klebs* *Anabaena sp. (unknown)* *anabaena sp. a* *Anabaena sp. b* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Gleoetrichia sp.* *Microcystis aeruginosa (Kutzing) Kutzing* *Oscillatoria sp.*	*Akinetes* *Aphanocapsa delicatissima W. and G.S. West* *Aphanocapsa incerta* *Aphanocapsa sp.* *Aphanothece sp.* *Chroococcus minutus (Kutzing) Nageli* *Chroococcus sp.* *Coelospaerium kuetzingianum Nageli* *Coelsphaerium sp.* *Cyanobium sp.* *Cyanodictyon sp.* *Gloeocapsa sp.* *Heterocysts* *Merismopedia sp.* *Phormidium sp* *Planktolyngbya limnetica* *Small blue greens* *Synechococcus sp.*
Pine Lake	*Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena skuja* *Aphanizomenon flos-aquae Ralfs and Born.* *Aphanizomenon sp.* *Gleoetrichia sp.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis sp.* *Microcystis viridis (Brunnthaler) Lemmermann* *Oscillatoria sp.* *Oscillatoria tenuis*	*Aphanocapsa delicatissima W. and G.S. West* *Aphanocapsa pulchra* *Aphanocapsa sp.* *Aphanothece sp.* *Chroococcus minutus (Kutzing) Nageli* *Chroococcus sp.* *Cyanobium sp.* *Cyanodictyon sp.* *Dictyosphaerium sp.* *Gomphosphaeria aponina Kutzing* *Gomphosphaeria sp.* *Lyngbya Birgei* *Phormidium sp* *Pseudoanabaena sp.* *Small blue greens* *Woronichinia robusta* *Woronichinia sp.*
Thunder Lake	*Anabaena flos-aquae (Lyngbye) Brebisson* *Anabaena spiroides Klebs* *Aphanizomenon flos-aquae Ralfs and Born.* *Microcystis aeruginosa (Kutzing) Kutzing* *Microcystis flos-aquae (Wittr.) Kirchn.* *Microcystis viridis (Brunnthaler) Lemmermann* *Microcystis wesenbergii* *Microcystis woronichinia* *Oscillatoria tenuis*	*Phormidium mucicola* *Woronichinia robusta*
Travers Reservoir	*Anabaena crassa* *Anabaena flos-aquae (Lyngbye) Brebisson*	*Lyngbya sp.[=Birgei]*

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Anabaena planctonica Brunnthaler
Anabaena sp.
Anabaena spiroides Klebs
Aphanizomenon flos-aquae Ralfs and Born.
Aphanizomenon klebhnii
Aphanizomenon sp.
Microcystis aeruginosa (Kutzing) Kutzing

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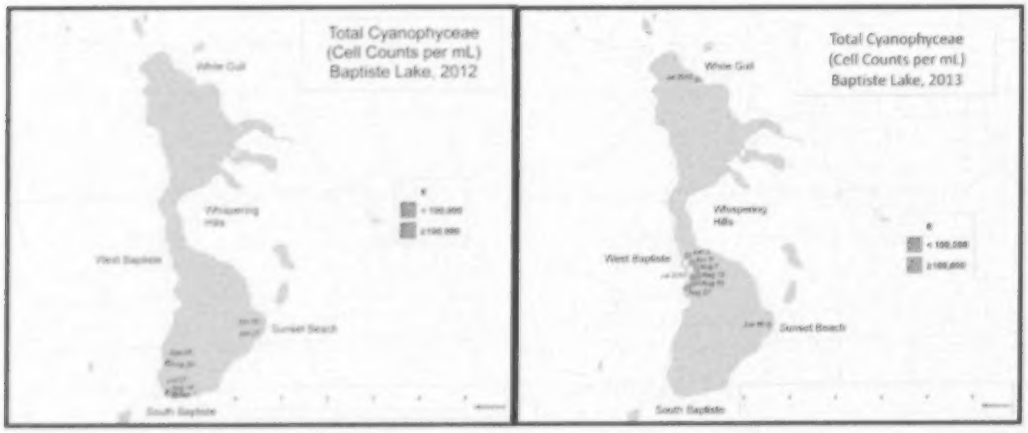
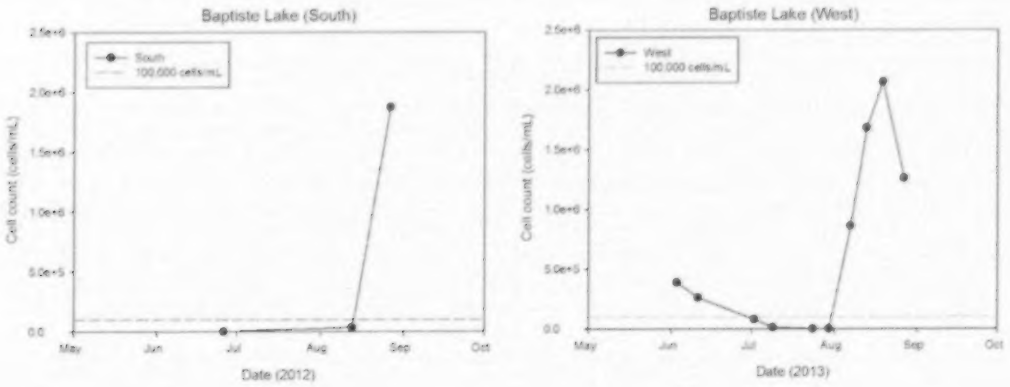
Baptiste Lake

2012: *Cyanobium sp.* and *Microcystis aeruginosa Kutzing* >100,000 cells/mL, respectively

2013: *Aphanizomenon flos-aquae Ralfs and Born., Aphanizomenon klebhnii, Aphanothece sp., Cyanobium sp., Gomphosphaeria aponina Kutzing, Microcystis aeruginosa (Kutzing) Kutzing, Microcystis flos-aquae (Wittr.) Kirchn., Oscillatoria sp., Woronichinia naegelianum (Unger) Elenk.* and *Woronichinia robusta* >100,000 cells/mL, respectively





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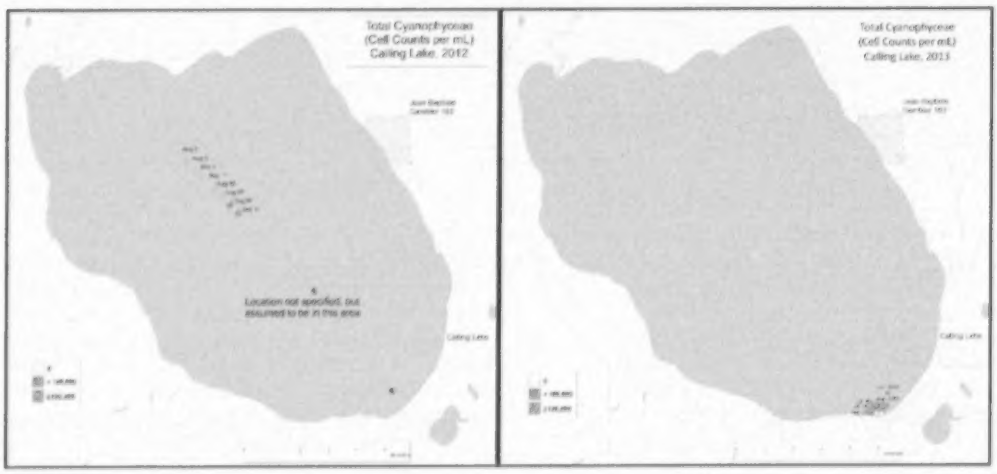
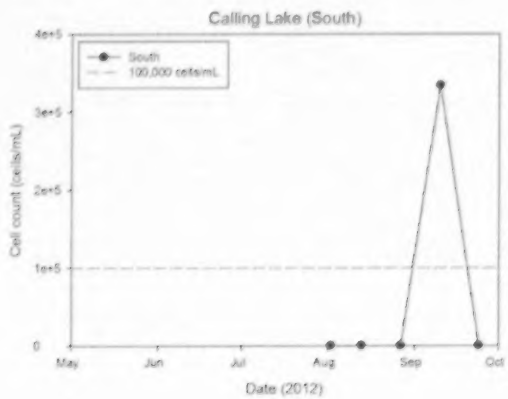
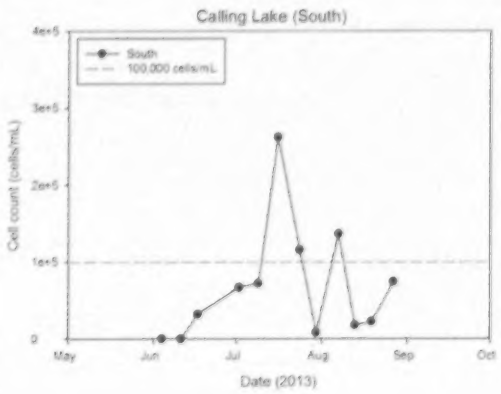
Calling Lake

2012: *Microcystis aeruginosa* >100,000 cells/mL

2013: *Aphanizomenon gracile (Lemmermann) Lemmermann* >100,000 cells/mL







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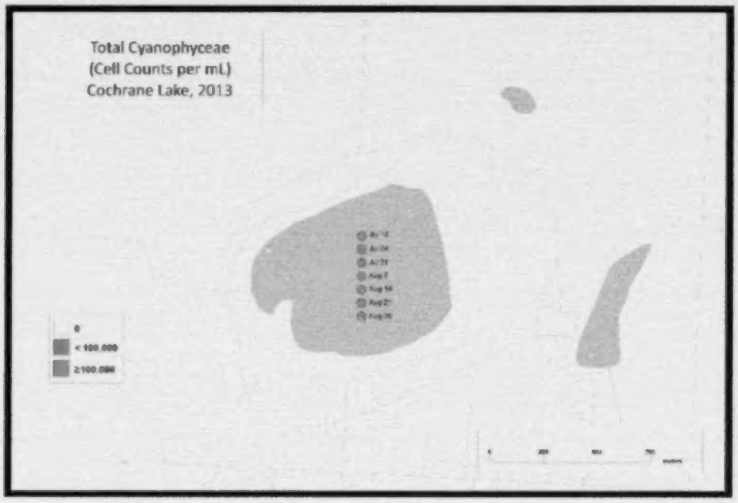
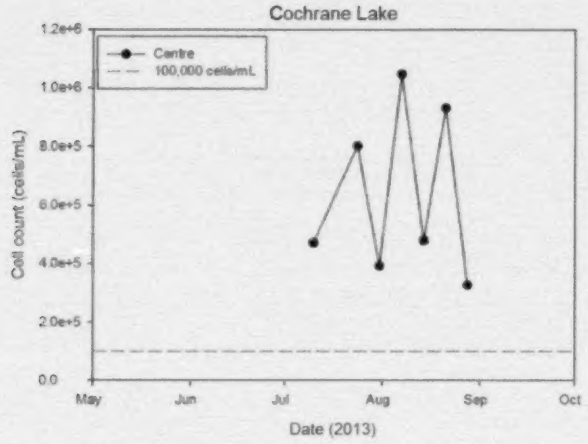
Cochrane Lake

2013: *Anabaena flos-aquae (Lyngbye) Brebisson*, *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis flos-aquae (Wittr.) Kirchn.* and *Microcystis viridis (Brunnthaler) Lemmermann* > 100,000 cells/mL, respectively





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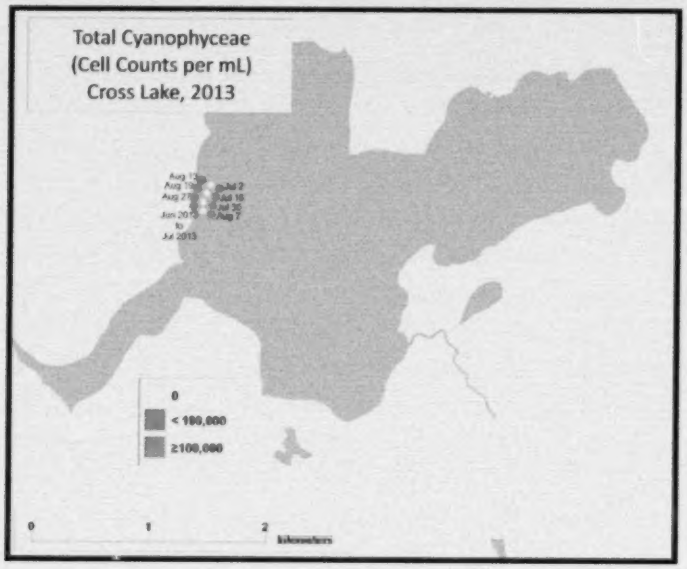
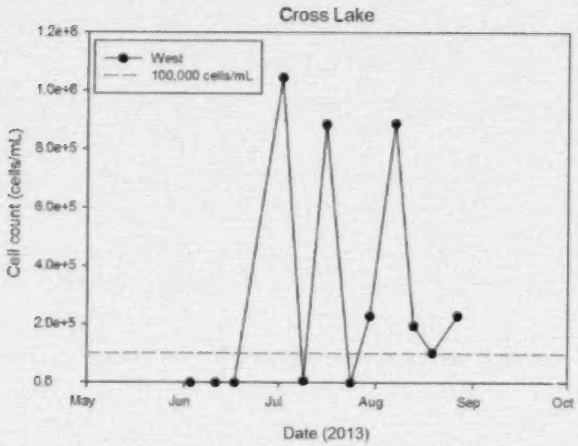
Cross Lake

2013: *Anabaena flos-aquae (Lyngbye) Brebisson*, *Anabaena sp. A*, *Anabaena spiroides Klebs*, *Aphanothece sp.* and *Gleoetrichia sp.* > 100,000 cells/mL, respectively





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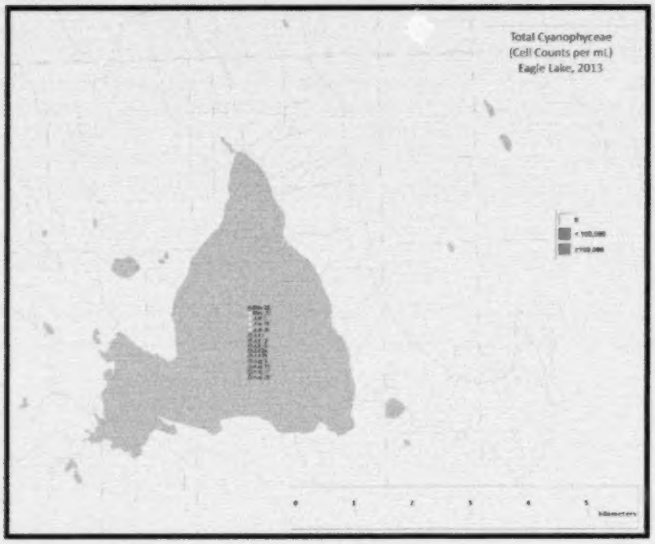
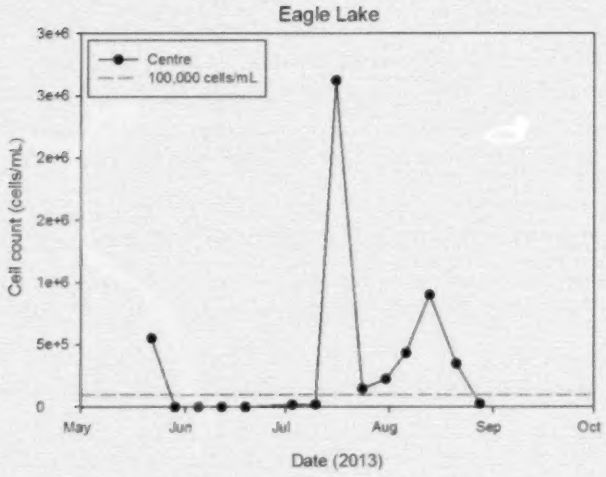
Eagle Lake

2013: *Aphanizomenon flos-aquae Ralfs and Born.*, *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis sp.* and *Pelogoea sp.* > 100,000 cells/mL, respectively





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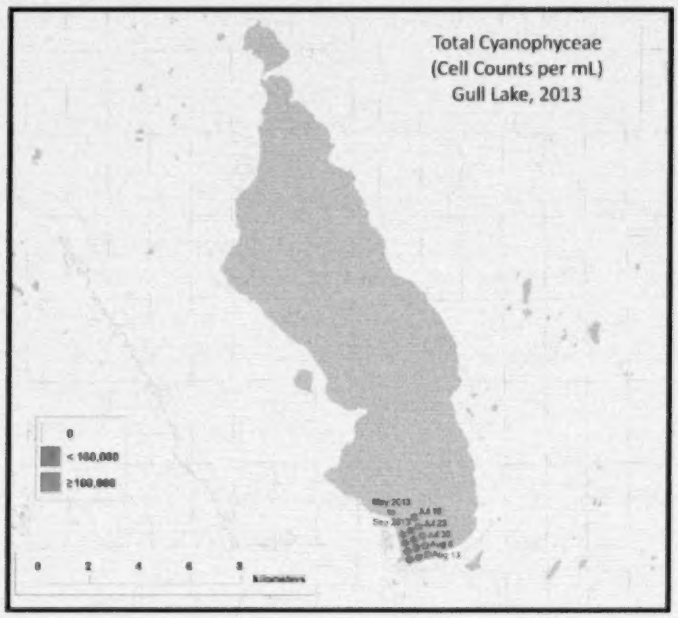
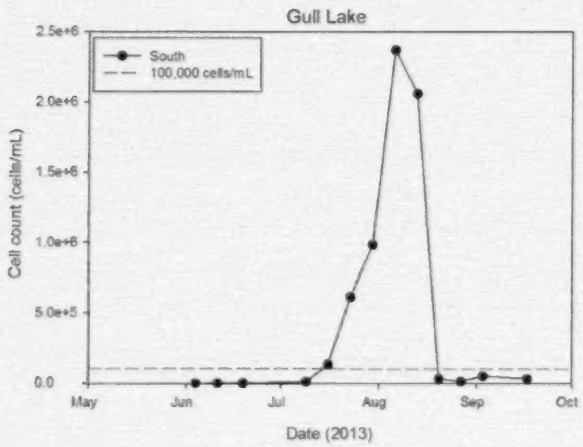
Gull Lake

2013: *Aphanocapsa sp.*, *Aphanothece sp.*, *Cyanobium sp.*, *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis flos-aquae (Wittr.) Kirchn.*, *Microcystis wesenbergii*, *Woronichinia naegelianum (Unger) Elenk.* and *Woronichinia robusta* > 100,000 cells/mL, respectively





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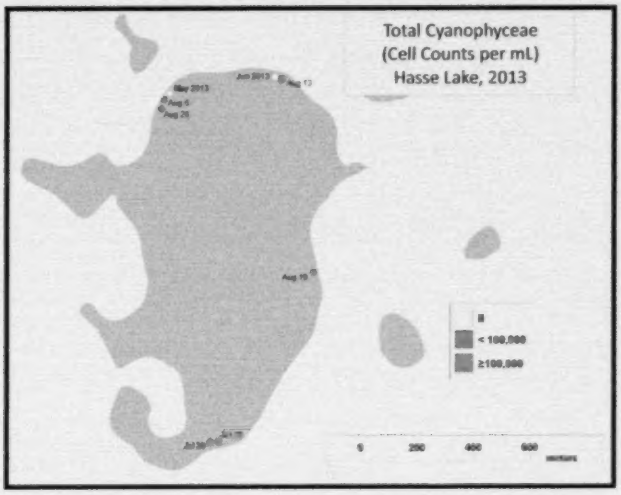
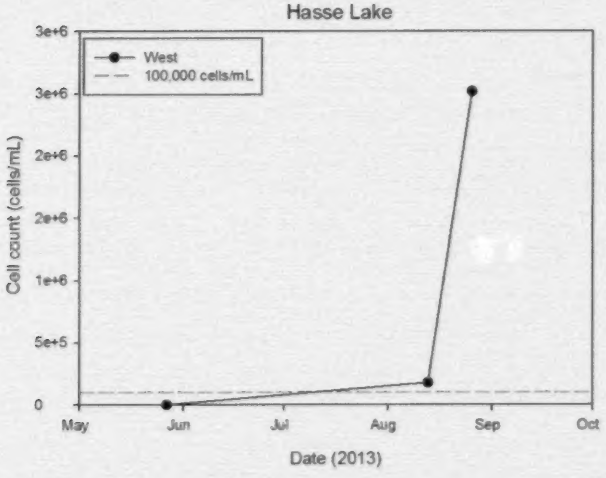
Hasse Lake

2013: *Aphanizomenon flos-aquae Ralfs and Born.*, *Microcystis aeruginosa (Kutzing) Kutzing* and *Woronichinia naegelianum (Unger) Elenk.* > 100,000 cells/mL, respectively





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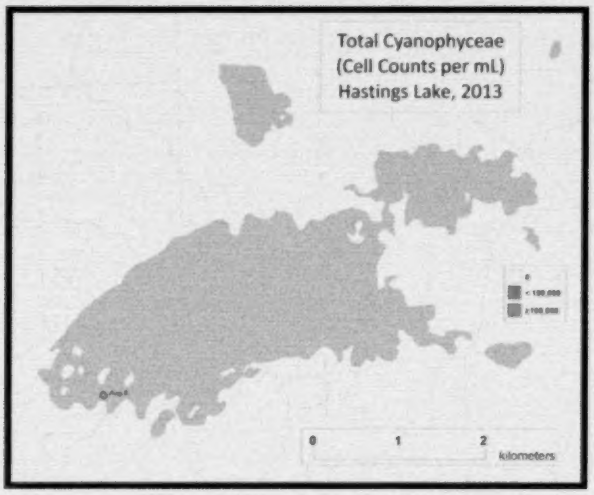
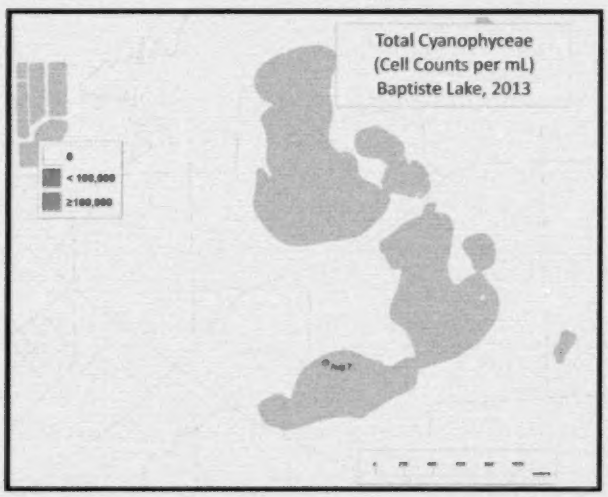
Hastings Lake

2013: *Microcystis wesenbergii* > 100,000 cells/mL



Haunted Lake

2013: *Microcystis aeruginosa (Kutzing) Kutzing* > 100,000 cells/mL



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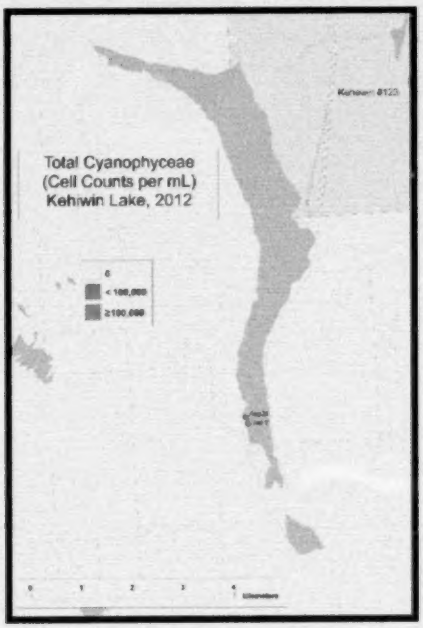
Kehewin Lake

2012: *Microcystis aeruginosa (Kutzing) Kutzing* >100,000 cells/mL



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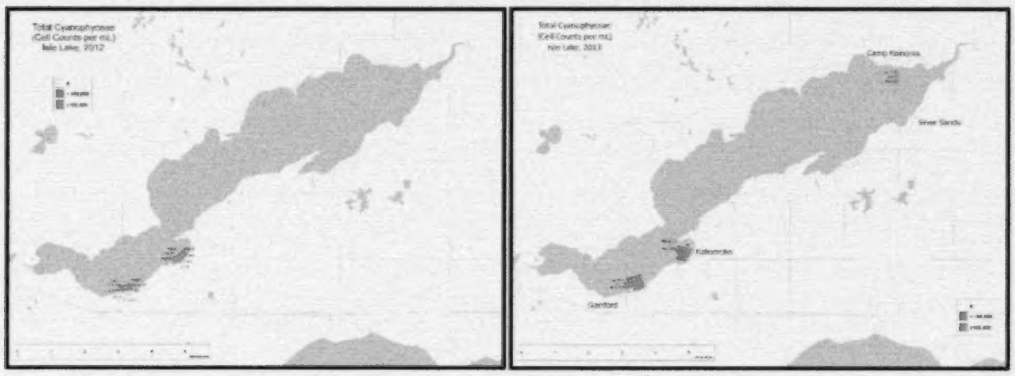
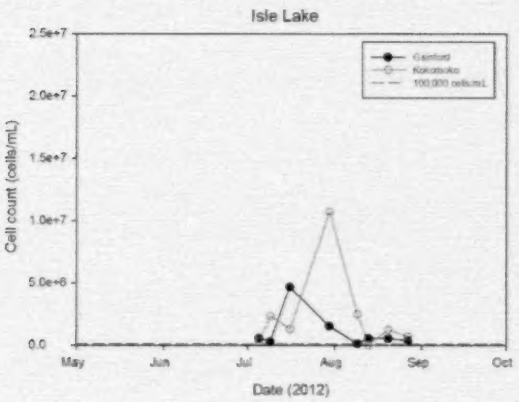
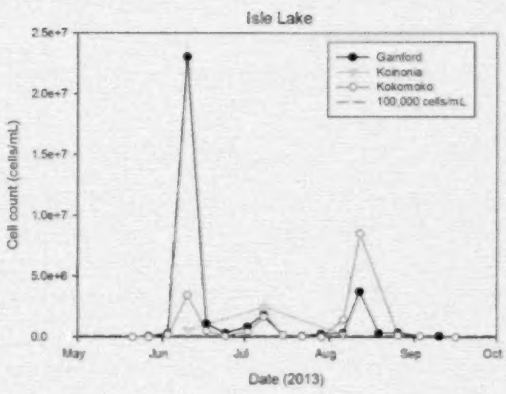
Isle Lake

2012: *Aphanizomenon flos-aquae Ralfs and Born.*, *Aphanizomenon insselshenkei*, *Aphaanizomenon sp.*, *Coelospaerium kuetzingianum Nageli*, *Cyanobium sp.*, *Cyanodictyon sp.*, *Microcystis aeruginosa (Kutzing) Kutzing* and *Microcystis wesenbergii*, *Synechococcus sp.* > 100,000 cells/mL, respectively

2013: *Anabaena flos-aquae (Lyngbye) Brebisson*, *Aphanizomenon flos-aquae Ralfs and Born.*, *Cyanodictyon sp.*, *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis flos-aquae (Wittr.) Kirchn.*, *Microcystis viridis (Brunnthaler) Lemmermann*, *Microcystis wesenbergii*, *Microcystis woronichinia*, *Oscillatoria sp.* and *Oscillatoria sp. A*, *Oscillatoria sp. A* > 100,000 cells/mL, respectively







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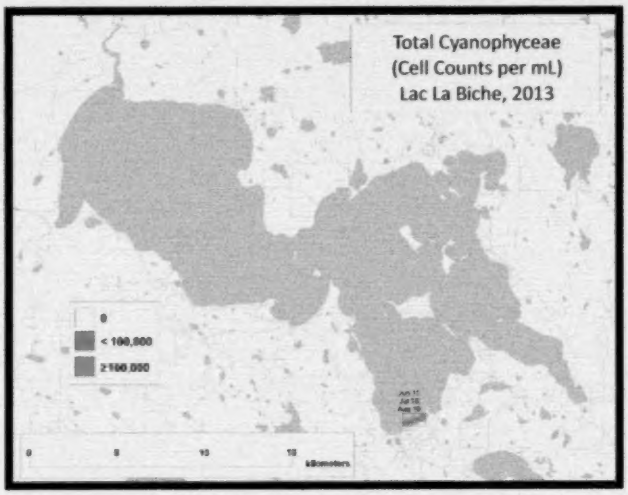
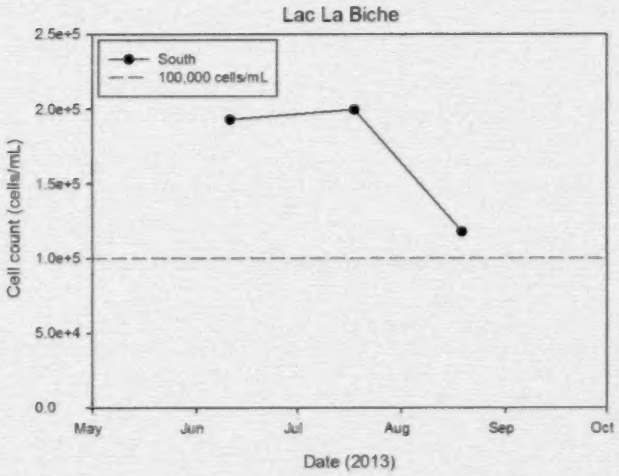
Lac La Biche

2013: *Anabaena flos-aquae*, *Anabaena flos-aquae (Lyngbye) Brebisson*, *Aphanocapsa delicatissima W. and G.S. West*, *Aphanocapsa sp.*, *Aphanothece sp.*, *Chroococcus minutus (Kutzing) Nageli*, *Chroococcus sp.*, *Coelospaerium kuetzingianum Nageli*, *Cyanobium sp.*, *Cyanodictyon sp.*, *Lyngbya sp.*, *Merismopedia sp.*, *Oscillatoria sp* and *Small blue greens* total adding all spp. >100,000 cells/mL





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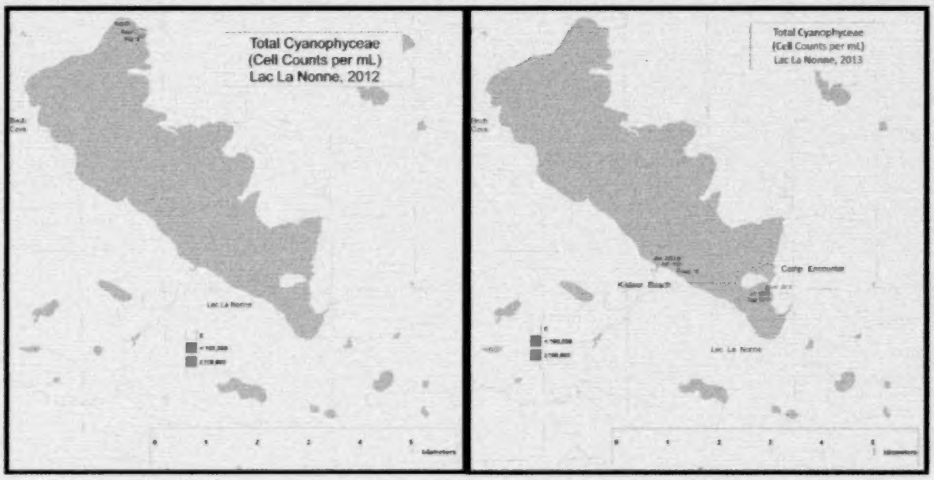
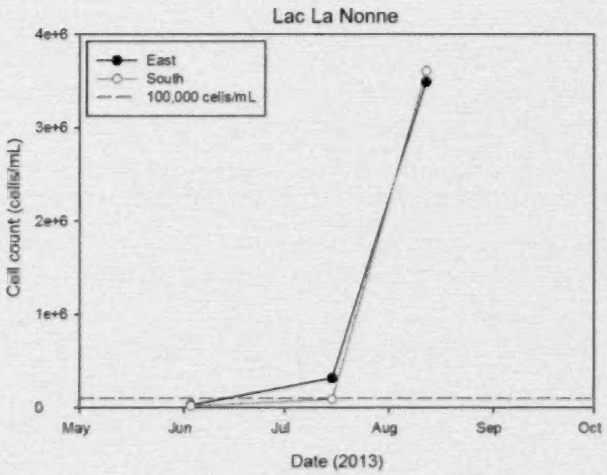
Lac La Nonne

2012: *Anabaena spiroides Klebs*, *Aphanocapsa sp.*, *Coelospaerium Naegelianum Unger*, *Cyanodictyon sp.*, *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis wesenbergii* and *Pseudoanabaena sp.* >100,000 cells/mL, respectively

2013: *Anabaena flos-aquae (Lyngbye) Brebisson*, *Anabaena spiroides Klebs* and *Microcystis aeruginosa (Kutzing) Kutzing* >100,000 cells/mL, respectively





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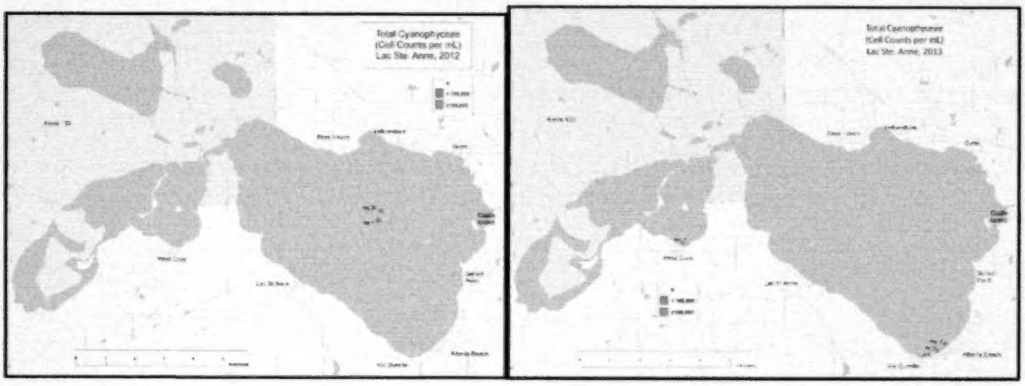
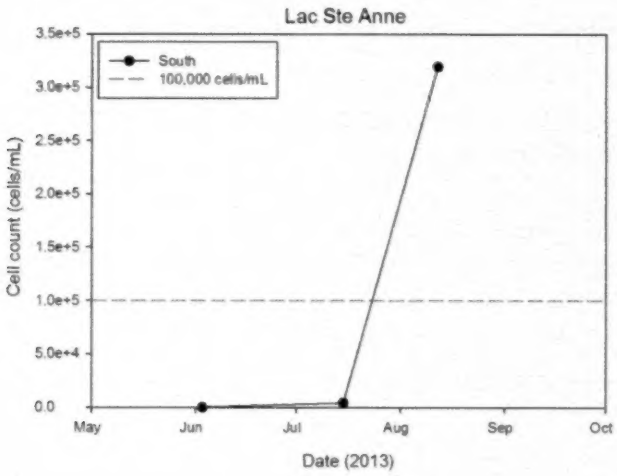
Lac Ste Anne

2012: *Cyanodictyon sp.*, *Gomphosphaeria aponina* and *Microcystis aeruginosa (Kutzing) Kutzing*

>100,000 cells/mL, respectively

2013: *Cyanodictyon sp.* >100,000 cells/mL, respectively





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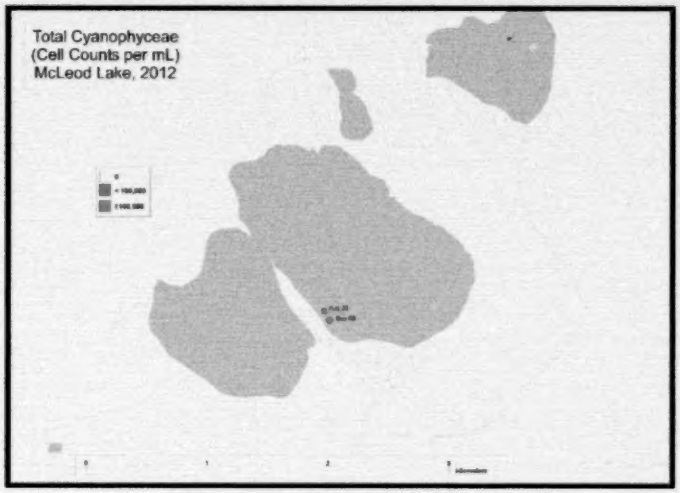
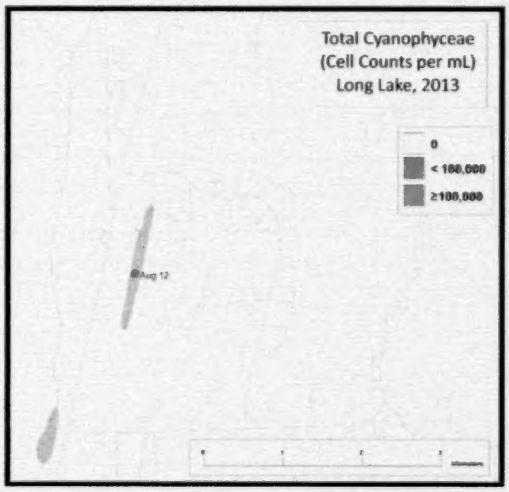
McLeod Lake

2012: *Microcystis flos-aquae (Wittr.) Kirchn.* >100,000 cells/mL



Long Lake

2013: *Microcystis woronichinia*, *Aphanizomenon flos-aquae Ralfs and Born.*, *Microcystis viridis (Brunnthaler) Lemmermann*, *Anabaena flos-aquae (Lyngbye) Brebisson*, *Woronichinia robusta* and *Anabaena perturbata* total adding all spp. < 100,000 cells/mL



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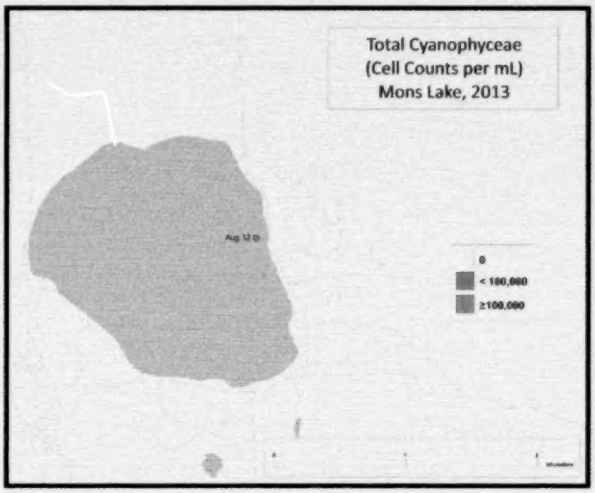
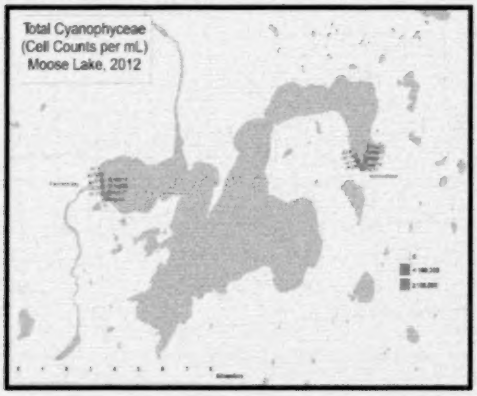
Mons Lake

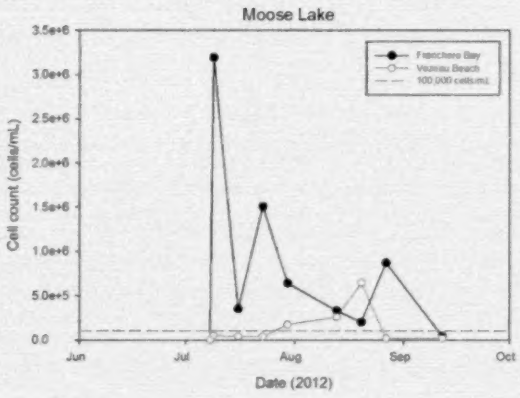
2013: *Cyanobium sp.* > 100,000 cells/mL



Moose Lake

2012: *Anabaena circinalis Rabenhorst*, *Cyanodictyon sp.*, *Gleoetrichia sp.*, *Microcystis aeruginosa (Kutzing) Kutzing* and *Microcystis viridis (Brunnthaler) Lemmermann*
>100,000 cells/mL, respectively





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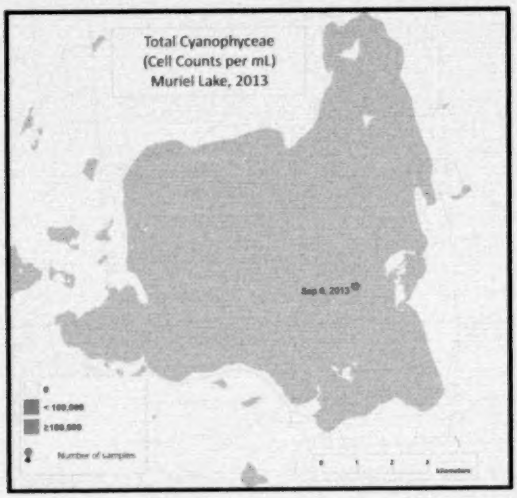
Muriel Lake

2013: *Microcystis aeruginosa (Kutzing) Kutzing*, *Cyanobium sp.* and *Microcystis flos-aquae (Wittr.) Kirchn.* > 100,000 cells/mL



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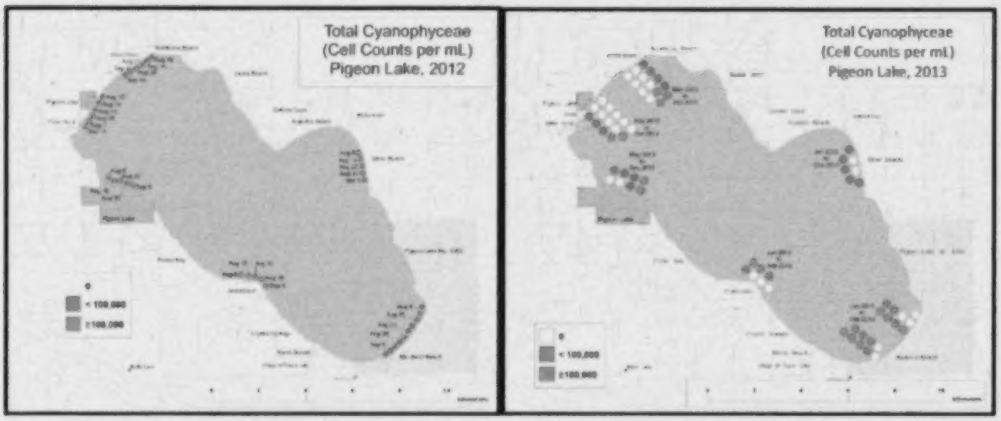
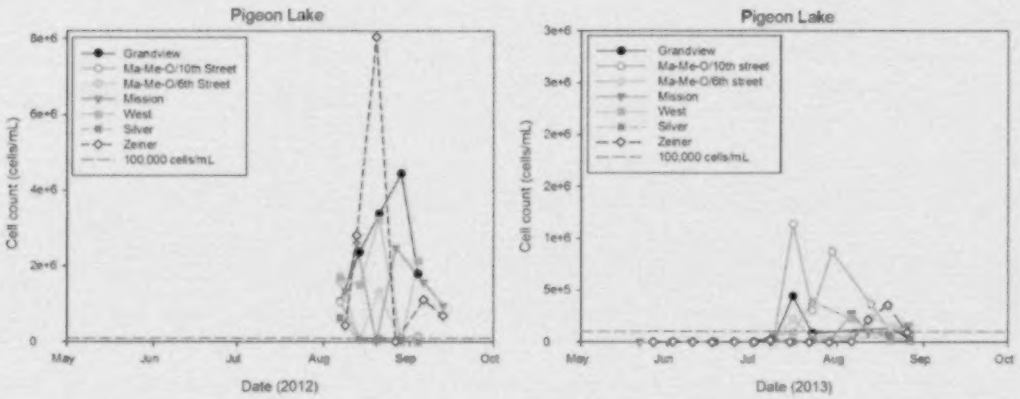
Pigeon Lake

2012: *Cyanobium sp.*, *Eucapsis sp*, *Gleoetrichia sp.*, *Microcystis aeruginosa (Kutzing) Kutzing*, *Planktolyngbya limnetica* and *Synechococcus sp.* > 100,000 cells/mL, respectively

2013: *Aphanizomenon flos-aquae Ralfs and Born.*, *Aphanocapsa delicatissima W. and G.S. West*, *Aphanocapsa incerta*, *Cyanodictyon sp.* and *Gleoetrichia sp.* > 100,000 cells/mL, respectively





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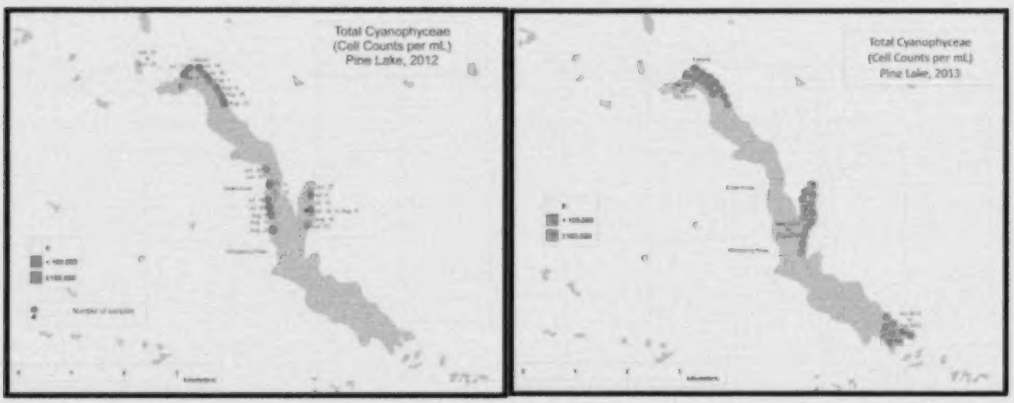
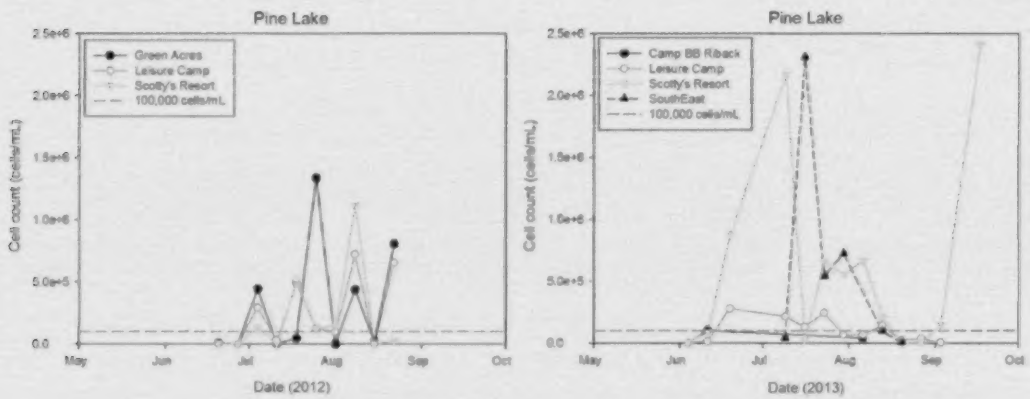
Pine Lake

2012: *Aphanizomenon flos-aquae Ralfs and Born.*, *Gomphosphaeria aponina Kutzing*, *Gomphosphaeria lacustris var. compacta (Lemmermann)*, *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis flos-aquae (Wittr.) Kirchn.*, *Microcystis viridis (Brunnthaler) Lemmermann*, *Oscillatoria tenuis* and *Synechococcus sp.* > 100,000 cells/mL, respectively

2013 : *Anabaena skuja*, *Aphanocapsa delicatissima W. and G.S. West*, *Aphanocapsa pulchra*, *Cyanobium sp.*, *Cyanodictyon sp.*, *Gleoetı hia sp.*, *Lyngbya Birgei*, *Microcystis aeruginosa (Kutzing) Kutzing* and *Woronichinia robusta* > 100,000 cells/mL, respectively





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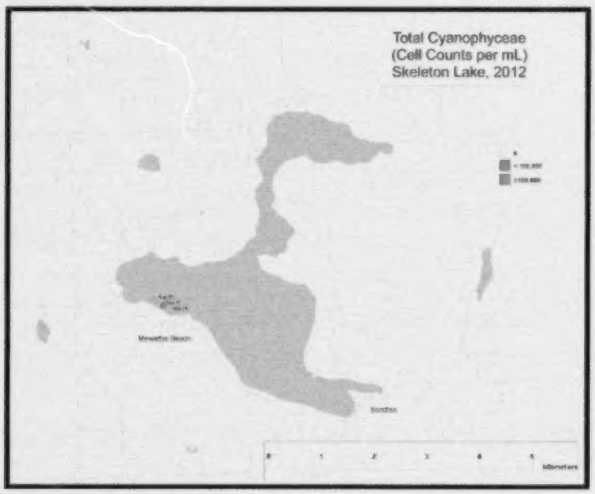
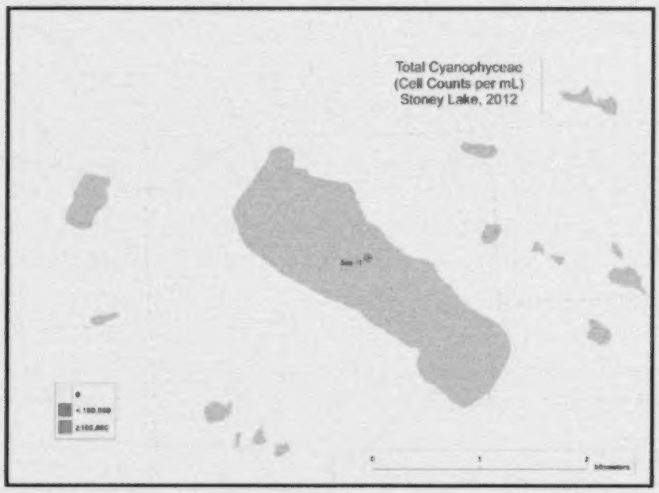
Skeleton Lake

2012: *Anabaena sp.*, *Coelsphaerium sp.*, *Gomphosphaeria natus Komarek and Hindak*, *Microcystis aeruginosa (Kutzing) Kutzing* and *Microcystis wesenbergii* total adding all spp. >100,000 cells/mL



Stoney Lake

2012: *Microcystis aeruginosa (Kutzing) Kutzing* >100,000 cells/mL



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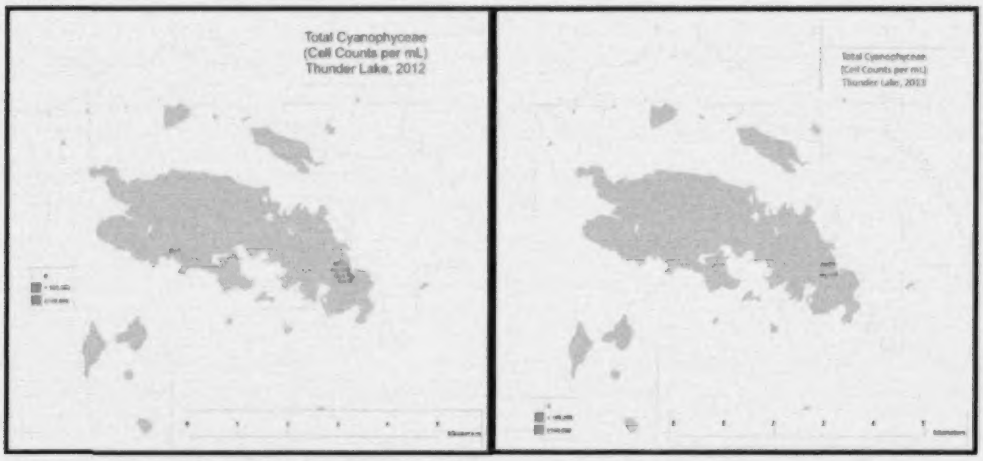
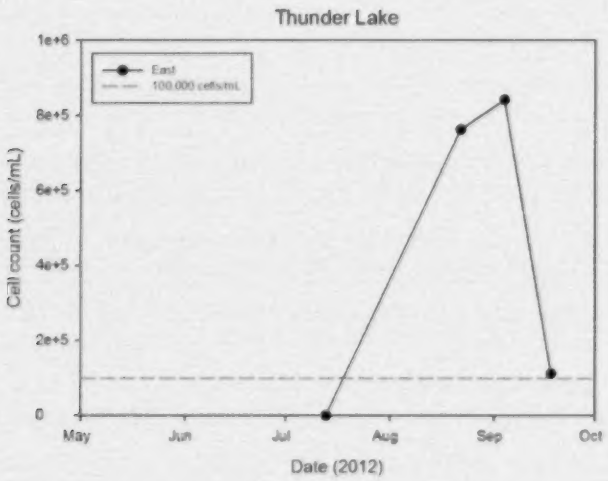
Thunder Lake

2012: *Merismopedia minima (Ehrenberg) Kutzing*, *Microcystis aeruginosa (Kutzing) Kutzing* and *Microcystis wesenbergii* > 100,000 cells/mL, respectively

2013: *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis flos-aquae (Wittr.) Kirchn.*, *Microcystis viridis (Brunnthaler) Lemmermann*, *Microcystis woronichinia*, *Phormidium mucicola* and *Woronichinia robusta* > 100,000 cells/mL, respectively





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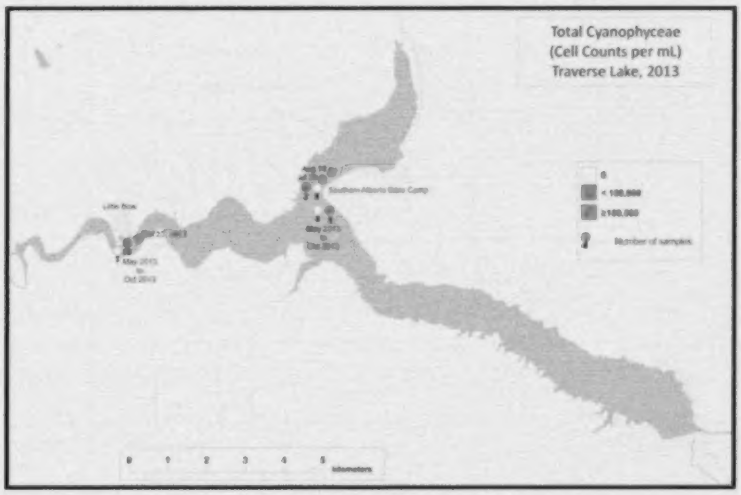
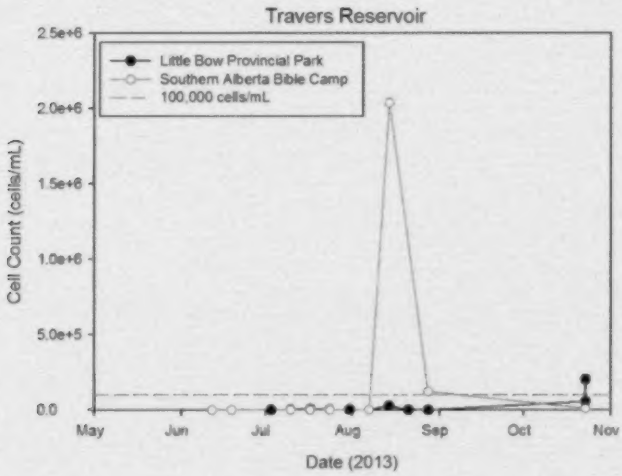
Travers Reservoir

2013: *Anabaena flos-aquae (Lyngbye) Brebisson* >100,000 cells/mL





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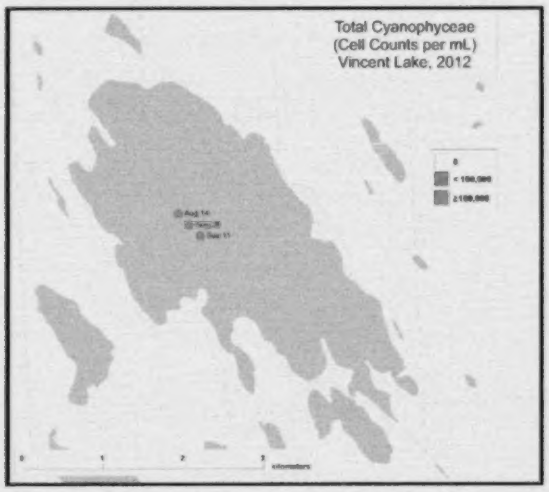
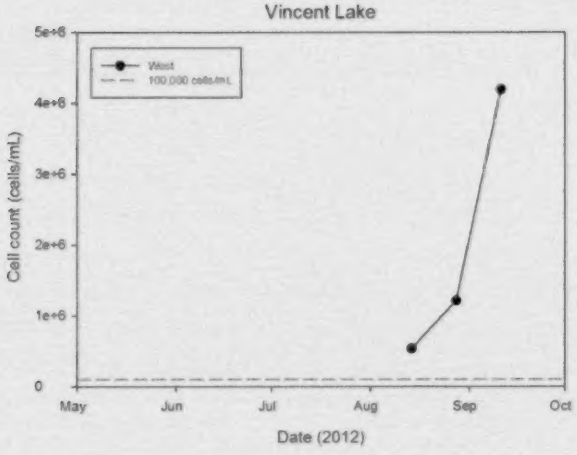
Vincent Lake

2012: *Microcystis aeruginosa (Kutzing) Kutzing*, *Microcystis wesenbergii*, *Oscillatoria sp.* >100,000 cells/mL, respectively





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Appendix D

Sensitivity and Specificity

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2×2 table of PPI compared with LC-MS/MS

		LC-MS/MS		
		>=20 µg/L	<20 µg/L	Total
PPI	positive (>=20 µg/L)	26	6	32
	negative (<20 µg/L)	3	1135	1138
Total		29	1141	1170
		LC-MS/MS		
		>=1.5 µg/L	<1.5 µg/L	Total
PPI	positive (>=1.5 µg/L)	170	47	217
	negative (<1.5 µg/L)	6	947	953
Total		176	994	1170

2×2 table of ELISA compared with LC-MS/MS

		LC-MS/MS		
		>=20 µg/L	<20 µg/L	Total
ELISA	positive (>=20 µg/L)	26	29	55
	negative (<20 µg/L)	0	579	579
Total		26	608	634
		LC-MS/MS		
		>=1.5 µg/L	<1.5 µg/L	Total
ELISA	positive (>=1.5 µg/L)	156	109	265
	negative (<1.5 µg/L)	0	369	369
Total		156	478	634

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Appendix E

Microcystin Levels in 2010 and 2011 by Using PPI Assay

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Total Sample Size and the Sample Size with Exceeding 20 µg MCYST/L (PPI)

	2010		2011	
	Total Sample	≥ 20 µg/L	Total Sample	≥ 20 µg/L
Auburn Bay	8		-	
Baptiste Lake	10		6	1
Beaver Lake	10		-	
Black Nugget Lake	-		2	
Bonavista Lake	8		-	
Buffalo Lake	43		42	
Calderon Acres	-		8	
Calling Lake	-		4	
Chaparral Lake	8		-	
Chestermere Lake	7		7	
Cross Lake	-		1	
Dried Meat Lake	9		-	
Eagle Lake	7		10	2
Elkwater Lake	-		7	
Ghost Lake	-		6	
Golden Eagle Pond	-		8	
Gregoire Lake	-		26	
Gull Lake	9		9	
Half Moon Lake	1		-	
Hasse Lake	9		10	
Hastings Lake	10		-	
Isle Lake (Lake Isle)	38	3	21	
Islet Lake	9		-	
Jackfish Lake	9		10	
Johnson Lake	-		-	
Kehewin Lake	-		-	
Lac La Biche	26		-	
Lac La Nonne	8	1	16	
Lac Sante	-		14	
Lac Ste Anne	7	1	8	
Little Beaver Lake	9		-	
Little Bow Lake Reservoir	20		8	
Maqua Lake	-		7	
McGregor Lake	7		7	
McKenzie Lake	8		-	
Midnapore Lake	8		-	
Miquelon Lake	9		-	
North Buck Lake	16		-	
Our Lady Queen of Peace Ranch	9		5	
Park Lake Reservoir	-		7	

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Pigeon Lake	60		67	2
Pine Lake	28	1	31	
Quarry Lake	-		-	
Rattlesnake Reservoir	-		7	
Ridge Reservoir	-		7	
Sikome Lake	6		-	
Skeleton Lake	16		-	
Spring Lake	8		-	
Steele Lake	10		-	
Sundance Lake	8		-	
Sylvan Lake	17		16	
Thunder Lake	7	2	8	5
Travers Reservoir	7		7	
Wabamun Lake	46		51	
Wizard Lake	10		17	4
Total	545	8	460	15

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Appendix F

Cell Density and/ or Microcystin Levels in 2012 and 2013

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Total Sample Size and the Sample Size with Exceeding Guidelines in 2012

	Total Sample	Cell Count ≥100,000 cells/mL	PPI ≥ 20 µg/L	ELISA >20 µg/l	LC/MS/MS ≥ 20 µg/L
40 Mile Reservoir	8				
Baptiste Lake	7	2		1	
Battle Lake	2			1	
Calderon Acres	9				
Calling Lake	8	1			
Cascade Pond	10				
Chestermere Lake	9				
Cow Lake	6				
Crane Lake	8				
Crimson Lake	6				
Cross Lake	6		1		1
Eagle Lake	14		3	5	3
Ghost Lake	9				
Gleniffer Lake	16				
Gregoire Lake	13			1	
Gull Lake	7				
Hasse Lake	12				
Hubbles Lake	11				
Isle Lake	18	15	1	6	1
Jackfish Lake	11				
Johnson Lake	10				
Kehewin Lake	5	1			
Lac La Biche	1				
Lac La Nonne	4	1	1	2	1
Lac Ste Anne	5	2			
McGregor Lake	10				
McLeod Lake	3	1			
Milk River	11				
Mink Lake	11				
Moonshine Lake	3		1	2	
Moose Lake	20	10	1	2	
Our Lady Queen of Peace Ranch	7				
Park Lake Reservoir	11				
Pigeon Lake	102	24		1	
Pine Lake	45	14			
Quarry Lake	10				
Rattlesnake Reservoir	9				
Skeleton Lake	3	1		1	1
Stoney Lake	3	1		1	
Sylvan Lake	8				
Thunder Lake	5	3	1		1
Tim Horton Children's Ranch	10				
Travers Reservoir	20				
Two Jack Lake	10				
Vincent Lake	5	3			
Wabamun Lake	37				
Wizard Lake	21				
Total	573	79	9	23	8

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Total Sample Size and the Sample Size with Exceeding Guidelines in 2013

	Total Sample	Cell Count ≥100,000 cells/mL	PPI ≥ 20 µg/L	ELISA >20 µg/l	LC/MS/MS ≥ 20 µg/L
40 Mile Reservoir	12				
Baptiste Lake	13	7			
Calderon Acres	14	2			
Calling Lake	12	3			
Cascade Pond	12				
Chestermere Lake	16				
Cochrane Lake	7	7	3	3	3
Cross Lake	12	7			
Eagle Lake	14	7	1	1	1
Fork Lake	3				
Ghost Lake	14				
Granum Lake	1				
Gregoire Lake	3				
Gull Lake	14	5			
Hanmore Lake	1				
Hasse Lake	8	6			
Hastings Lake	1	1			
Haunted Lake	1	1	1	1	1
Hawrelak Pavilion Park	6				
Hubbles Lake	8	2			
Isle Lake	41	24	1	4	1
Jackfish Lake	4				
Johnson Lake	13				
Lac La Biche	3	3			
Lac La Nonne	6	3	2	2	2
Lac Ste Anne	4	1			
Long Lake	1				
McGregor Lake	14				
Milk River Ridge	13				
Mink Lake	4	1			
Mons Lake	1	1			
Moose Lake	8	8			
Muriel Lake	1	1	1	1	
Owners: Condominium Corp	11				
Park Lake	13	1			
Pigeon Lake	101	16			
Pine Lake	36	17			
Quarry Lake	14				
Rattlesnake Reservoir	12				
Ridge Park	13				
Skeleton Lake	3				
Sylvan Lake	29	1			
Thunder Lake	3	1			
Tim Horton Children's	14				

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Ranch					
Travers Reservoir	35	3	1		
Twin Valley Reservoir	2				
Two Jack Lake	13				
Wabamun Lake	13				
Wizard Lake	15	2			
Total	612	131	10	12	8

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Appendix G

Cyanobacteria Genera and their Cyanotoxin

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Toxins and Toxin-producing Cyanobacterial Genera

Structure	Cyanotoxin	Primary target organ in mammals	Cyanobacteria genera
Cyclic peptides	Microcystins	Liver	*Microcystis*, *Anabaena*, *Planktothrix* (Oscillatoria), *Nostoc*, *Hapalosiphon*, *Anabaenopsis*
Alkaloids	Nodularins	Liver	*Nodularia*
	Anatoxin-a	Nerve synapse	*Anabaena*, *Planktothrix* (Oscillatoria), *Aphanizomenon*
	Anatoxin-a(S)	Nerve synapse	*Anabaena*
	Cylindrospermopsins	Liver	*Cylindrospermopsis*, *Aphanizomenon*, *Umezakia*
	Lyngbyatoxin-a	Skin, gastro-intestinal tract	*Lyngbya*
	Saxitoxins	Nerve axons	*Anabaena*, *Aphanizomenon*, *Lyngbya*, *Cylindrospermopsis*
	Lipopolysaccharides	Potential irritant; affects any exposed tissue	*all*
Polyketides	Aplysiatoxins	Skin	*Lyngbya*, *Schizothrix*, *Planktothrix* (Oscillatoria)

Five MCYST Variants and Toxin-producing Species

Variant	Species	Variant	Species
MC-LR	*Anabaena* *Anabaenopsis* *Microcystis* *Nostoc*	MC-YR	*Microcystis aeruginosa* *Microcystis viridis* *Hapalosiphon spp.*
MC-RR	*Anabaena sp.* *Microcystis aeruginosa* *Microcystis viridis* *Oscillatoria agardhii*	MC-LW	*Microcystis aeruginosa*
		MC-LF	*Microcystis aeruginosa*

*Sources: (de Figueiredo et al 2004, WHO 1999)

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Appendix H

Advisory Signage for Public

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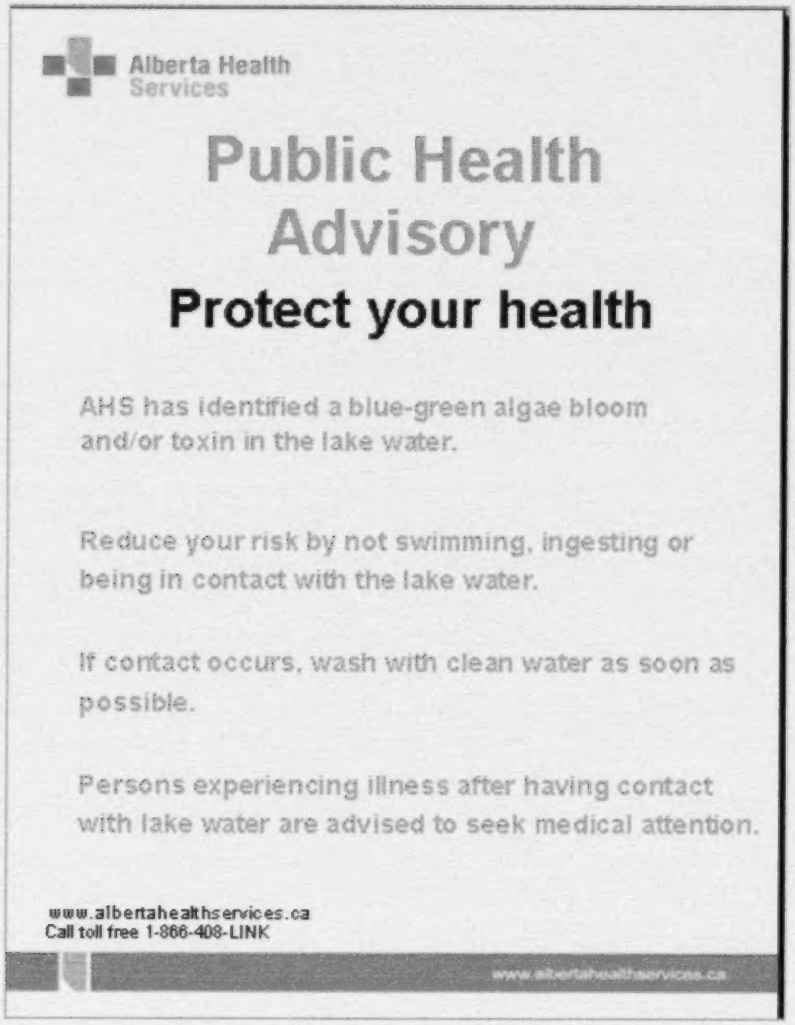
Advisory Signage


Alberta Health Services
Public Health Advisory
Protect your health
AHS has identified a blue-green algae bloom and/or toxin in the lake water.
Reduce your risk by not swimming, ingesting or being in contact with the lake water.
If contact occurs, wash with clean water as soon as possible.
Persons experiencing illness after having contact with lake water are advised to seek medical attention.
www.albertahealthservices.ca
Call toll free 1-866-408-LINK
www.albertahealthservices.ca

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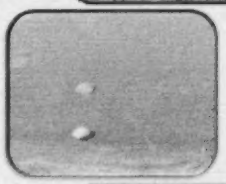
Public Information Blue-Green Algae

What is blue-green algae?

- Also called "cyanobacteria" and is naturally occurring in most Alberta lakes.
- Can produce a very potent toxin that can present a health risk to humans and animals that come in contact with algal blooms.

What to look for:

- A bluish-green scum with the appearance of pea-soup on the water surface with a musty odor.
- Algal blooms are unpredictable and can develop very quickly and move to other areas of the lake.



Public Health concerns:

- Contact can cause eye, ear, skin irritation, rashes and allergic reactions.
- Consuming contaminated water can cause nausea, diarrhea, vomiting, stomach cramps, liver damage and, in high concentrations, severe illness, including death.

How to Protect Yourself:

- Avoid swimming in water containing blue-green algae.
- Do not drink lake water. Boiling water does not remove or destroy toxins.
- Avoid contact with blue-green algae that has washed up on shorelines.
- Keep children, pets and livestock away from water contaminated with blue-green algae.

Contact for further information:

Alberta Health Services 1-866-408-LINK (5465)

Alberta Health Services

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